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Optimizing lysine and methionine nutrition during the periparturient and postparturient periods

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OPTIMIZING LYSINE AND METHIONINE NUTRITION DURING THE PERIPARTURIENT AND POSTPARTURIENT PERIODS

BY

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DISSERTATION

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in

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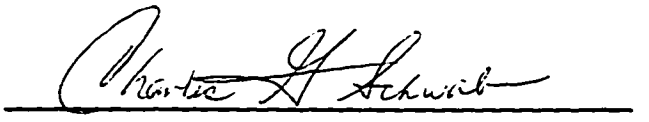

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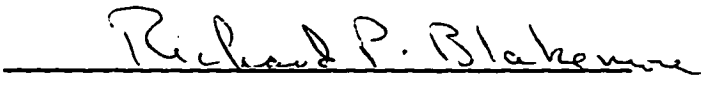

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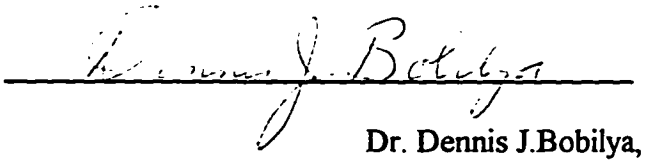

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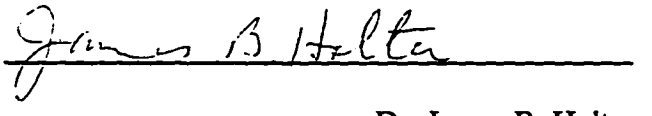

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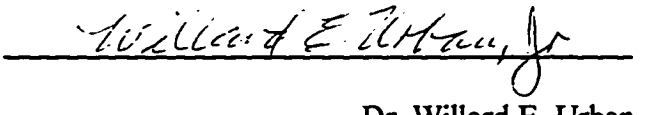

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DEDICATION

This dissertation is dedicated to my future husband, Steven R. Carson, for all his love, support, and patience.

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ABSTRACT

OPTIMIZING LYSINE AND METHIONINE NUTRITION IN THE PERIPARTURIENT AND POSTPARTURIENT PERIODS

by
Vicky M. Wasserstrom
University of New Hampshire, December, 1996

The primary objectives of experiment 1 were to determine the effects of changes in feed intake associated with the periparturient period (late gestation to early lactation) on passage of nitrogen fractions and amino acids to the small intestine and to determine if changes occurred in the profile of amino acids presented to the small intestine that could not be explained by diet composition. A secondary objective was to obtain preliminary data on changes in plasma concentrations of nonesterified fatty acids during the periparturient period when cows were fed nutrient-dense diet prepartum diets. Beginning 14 days before expected calving, 10 multiparous cows, five with ruminal and duodenal cannula, were fed prepartum diets containing (% of dry matter) 33.2 corn silage, 22.1 haycrop silage, 29.9 ground shelled corn, 11.6 solvent-extracted soybean meal, 0.7 blood meal, 0.7 tallow, and 1.9 mineral mix. On the day of calving through 12 days of lactation cows were fed a diet of (% of dry matter) 20.6 corn silage, 20.6 haycrop silage, 35.9 ground shelled corn, 17.0 soybean meal, 1.4 blood meal, 1.4 tallow, and 4.0 mineral mix. Feeding a nutrient-dense prepartum diet with the same ingredients as the postpartum diets stabilized rumen fermentation during the periparturient period and minimized the decrease in feed intake that accompanies parturition. Similar proportional flows of bacterial N and non-ammonia non-microbial N to the small intestine throughout the periparturient period resulted in a stable profile of amino acids in duodenal

protein prepartum diets than cows fed the low ruminally undegradable protein diets and greater for cows fed the lysine and methionine supplemented lactation diet that cows fed the unsupplemented diet. Increases in milk protein with postpartum lysine and methionine were greatest when high ruminally undegradable protein prepartum diets were fed vs. when low ruminally undegradable protein prepartum diets were fed and when amino acids were not included in the prepartum diets vs. when they were included in the prepartum diets. Concentration of ruminally undegradable protein and concentrations of lysine and methionine in ruminally undegradable protein of prepartum diets both appear to affect the milk protein content response of early lactation cows to supplemental lysine and methionine.

The primary objectives of experiment 3 were to determine the effects of, and interaction between, feeding greater amounts of ruminally undegradable protein and supplemental lysine and methionine during the last 3 weeks of gestation and supplemental lysine and methionine during the first 10 weeks of lactation on selected blood metabolites and hormones when soybean products provided all of the supplemental protein. The same cows used in experiment 2 were used in this experiment. Blood samples were taken daily beginning 10 days before calving through 7 days of lactation, after which samples were taken three times weekly for weeks 2 through 10 of lactation. Urine sampling followed the same scheme as blood samples. Concentrations of ruminally undegradable protein increased prepartum insulin, increased postpartum glucose, and reduced urine ketone concentrations.

INTRODUCTION

The fundamental goal of late gestation nutrition is to allow the dairy cow to make the transition from pregnancy to lactation without complications and with feed intakes that optimize milk synthesis. Therefore, the focus of many recent experiments has been to acquire a better understanding of organic nutrient metabolism during the transition from late gestation to early lactation and to fine-tune late gestation diets to minimize postpartum metabolic disorders while maximizing subsequent lactational performance.

Several experiments have indicated that increasing the concentration of ruminally undegradable protein prepartum diets will increase dry matter intake both pre- and postpartum, alter blood metabolite concentrations pre- and postpartum, and alter hormone concentrations associated with the transition from late gestation to early lactation. It is generally accepted that lysine and methionine are the two most limiting amino acids in lactating cows fed corn-based diets and therefore, it is expected that they also are the two most limiting amino acids in periparturient cows fed corn-based diets. Therefore, improving lysine and methionine nutrition during late gestation may play a role in subsequent lactational performance and altering concentrations of blood hormones and metabolites.

The objectives of the research presented in this thesis were to examine the effects of the transition period on passage of nitrogen fractions and amino acids to the small intestine. With this information, it was desired to assess the influence of changes in prepartum dietary protein concentration and lysine and methionine supplementation on subsequent lactational and blood metabolite (glucose, nonesterified fatty acids, growth hormone, insulin, and prolactin) responses to postpartum lysine and methionine supplementation.

CHAPTER I

REVIEW OF LITERATURE

Traditionally, early lactation has been characterized as a period of negative energy balance in which caloric intake does not meet the productive needs of the high producing cow. The result is the mobilization of adipose tissue reserves to meet the energy requirements for milk production and maintenance (3). In addition to energy, protein seems to be a major limiting nutrient for lactational performance, and labile protein reserves often are overlooked when addressing early lactation performance (3, 65) Availability of nutrient reserves to support early lactation, during a period of insufficient dry matter intake (**DMI**), depends on the diet fed during late gestation. Energy status has been the primary focus of studies with cows in late gestation. However, recent work (13, 17, 65) indicates that feeding late gestation cows higher crude protein (**CP**) diets results in greater milk production in early lactation and reduced incidence of post-calving metabolic disorders.

Protein requirements, particularly during late gestation, are not well defined. Recent studies (6, 13, 65) indicated that current models (i.e. NRC, 43) underestimate the net gestational protein requirement during late gestation. The NRC (43) recommendation for a 600-kg cow in late gestation is 100 g/d; this model accounts for protein deposition in fetal membranes, fluids, and uterine growth. However, when this estimate is compared with the net protein required for actual conceptus composition (6), it is evident that the model

underestimates the protein requirement by about 300 g/d. Therefore, feeding late gestation cows to NRC (43) recommendations may induce prepartal protein mobilization, whereas feeding a higher crude protein diet with the inclusion of some high quality ruminally undegradable protein (**RUP**) sources to better support conceptus development in late gestation may improve postpartum performance by minimizing depletion of maternal labile protein reserves (65).

Metabolic Adaptations in Late Gestation

Alterations in maternal carbohydrate, protein, and lipid metabolism occur in late gestation to accommodate the substantial glucose and amino acid (**AA**) needs of maternal and conceptus tissues (7). The energy and nitrogen requirements of the conceptus are met almost exclusively by glucose and AA. Therefore, alterations in lipid metabolism meet maternal energy needs, while alterations in carbohydrate and protein metabolism meet both conceptus and maternal needs (6).

Enhanced gluconeogenesis is the most significant alteration in maternal carbohydrate metabolism that occurs during late pregnancy, even when animals are well fed. Steel and Leng (62) reported increased rates of whole-body gluconeogenesis in ewes during late pregnancy as compared to non-pregnant ewes. Some of the increase in glucose production was attributed to increased DMI when ewes were fed ad libitum. However, increased rates of glucose production also occurred in ewes in which intake was restricted (46, 62). Greater hepatic gluconeogenesis in ad libitum-fed as compared to restricted-fed ewes appeared to be the result of increased hepatic uptake of gluconeogenic substrate, including AA, glycerol, and lactate (62). Reynolds et al (49) indicated that there was a pregnancy-induced reduction in

glucose uptake and use by peripheral tissue. In agreement with Reynolds et al (49), Faulkner and Pollock (20) reported a reduction in glucose uptake in cultured adipose tissue cells from pregnant sheep compared with cells from non-pregnant sheep (20). The reduction in glucose use by peripheral tissues appears to be an effort to spare glucose for use by the conceptus (49).

The increase in gluconeogenesis in late gestation may also prevent the accumulation of lipid in the liver; blood glucose concentration and liver glycogen concentration prior to parturition are inversely related to liver triacylglycerol (TG) accumulation. Studer et al (64) reported a 43 % reduction in liver TG accumulation in dairy cows between d 17 prior to calving and d 1 and 21 postpartum when blood glucose concentration was elevated due to propylene glycol administration (1-L per day beginning 10 d before, until the day of calving). The positive effect of propylene glycol administration prepartum not only increased blood glucose concentration, but also decreased nonesterified fatty acid (NEFA) concentration and increased insulin concentration in blood. Increased lactation responses and decreased occurrence of metabolic disorders following calving as a result of propylene glycol administration were attributed to the alterations in blood glucose, insulin, and NEFA. In agreement with Studer et al (64), Veenhuizen et al (67) reported cows experimentally induced to develop ketosis during early lactation, had greater liver TG and reduced plasma glucose concentrations. In a similar group of cows induced to develop ketosis but duodenally infused with 484 g/d glucose, liver TG concentration was reduced, while plasma glucose and liver glycogen concentrations were increased compared to cows with ketosis. They concluded that duodenal infusions of glucose prevented the accumulation of TG in liver since plasma NEFA

concentration was similar in both groups (67).

The maternal adaptations in carbohydrate metabolism in late pregnancy are concurrent with alterations in lipid metabolism. Namely, increases in nutrient demand appear to increase the mobilization of adipose tissue reserves. Mobilization of lipid is indicated by elevated NEFA and ketone concentrations in plasma, even in animals fed to predicted energy requirements for conceptus growth and maintenance of nonuterine tissues (7, 8, 46, 66). This trend becomes more evident close to term and is exaggerated when feed intake declines near parturition (8). Plasma concentration of NEFA is related directly to uptake and oxidation of NEFA by the liver in ruminants (2). Additionally, the incomplete oxidation of NEFA in the liver accounts for the increase in hepatic ketogenesis and concentration of plasma 3-hydroxy butyrate (2). Hepatic accumulation of lipid occurs when uptake and rate of fatty acid esterification exceeds the rate of secretion of TG rich, very low density lipoprotein (VLDL). The difference between TG synthesis in the liver and VLDL export can result in moderate to severe hepatic lipidosis. Occurrence of hepatic lipidosis is about 30% in high yielding periparturient cows (2). Bertics et al (8) reported that accumulation of TG in the liver doubled between d 17 and d 2 prepartum and then increased two-fold again to peak at calving. The development of hepatic lipidosis during the periparturient period appears to increase the incidence of ketosis and reduce milk production in early lactation (24).

The effects of late pregnancy on quantitative AA metabolism have not been studied in ruminants. However, results from an experiment to measure hepatic protein synthesis at four stages of the reproduction-lactation cycle with isotope dilution and repeated liver biopsies, revealed a two-fold increase in hepatic protein synthesis from d 47 to d 9 before

calving and a further increase (about 10%) from d 9 before calving to d 6 of lactation despite unchanged or declining protein intake (7, 22). Furthermore, it appears that proteolytic activity and AA release from maternal muscle increases in late pregnancy (7). The net release of nitrogen from skeletal muscle occurs to supply the liver with AA for protein synthesis and gluconeogenesis. McNeill et al (42), using serial slaughter and calculated nitrogen efficiency values, reported a substantial mobilization of nitrogen from carcass tissues during late pregnancy when ewes were fed a low (8%) CP diet compared with medium (12%) and high (15%) CP diets. They provided evidence indicating that skeletal muscle was the primary source of labile protein and that the net flux of AA to and from skeletal muscle was quite sensitive to protein nutrition in late pregnancy. They estimated that skeletal muscle could contribute as much as 14 % of the AA required to support maternal and fetal requirements. They concluded that the maintenance or enhancement of skeletal muscle protein reserves by feeding greater dietary concentrations of protein during late pregnancy may be advantageous to ensure the availability of an endogenous supply of AA. The endogenous AA would then be available to supplement the supply of AA from metabolizable protein for milk protein synthesis and for hepatic gluconeogenesis during early lactation (42).

The metabolic adaptations of late pregnancy appear to occur to provide glucose and AA for conceptus metabolism. Unlike glucose, fetal uptake of AA occurs by active transport across the placenta; therefore, uptake is independent of changes in maternal blood concentration and protein undernutrition of the mother has little effect on fetal uptake of AA (6). Amino acid needs of the growing fetus are difficult to quantify because of their role in metabolic functions other than protein synthesis. Amino acids play an important role as

interorgan shuttles for nitrogen and carbon, as well as contributors to carbon accretion in the forms of pyruvate and α-ketoglutarate (1). Another crucial role for AA in the fetus is to serve as metabolic fuel, especially when there is a deficit of glucose due to insufficient maternal feed intake (1).

Endocrine Adaptations during the Transition Period

Periparturient metabolic adaptations are initiated in late pregnancy and amplified in early lactation. The most active and perhaps important tissues are liver and adipose. The regulation of metabolic adaptations is controlled by hormones such as insulin, estradiol, prolactin, and growth hormone (7). Resulting changes in hormone concentrations increase NEFA release from adipose tissue, increase glucose, and increase protein synthesis in the liver, and decrease glucose and AA utilization by extrahepatic and extramammary tissues.

Insulin. The insulin resistance of late pregnancy that typically occurs in humans and laboratory animals (39) also occurs in ruminants (7). Insulin resistance is characterized by a decreased tissue sensitivity to insulin, manifested as decreased whole-body glucose utilization (45). Despite adequate insulin concentration, adipose tissue response is decreased (45), resulting in fatty acid mobilization. Additionally, fatty acid mobilization during late pregnancy appears to be facilitated by the inability of insulin to stimulate lipogenesis. Lipogenesis opposes lipolysis; therefore, decreased lipogenesis increases lipolysis and elevates plasma NEFA and glycerol concentrations. Altered glucose utilization during insulin resistance appears to be related to its role in providing NADPH and glycerol-3-P for fatty acid esterification (7).

Studer et al (64) reported increased plasma insulin concentration of prepartum cows

by oral dosing with 1-L per day of propylene glycol. Dosing began 10 d prepartum and continued until parturition. Liver biopsies were taken at d 17 prior to parturition and d 1 and 21 of lactation. The increase in plasma insulin was attributed to an increase in ruminal propionate, since peak concentration of plasma insulin occurred before peak glucose. Propionate is a stimulant of pancreatic insulin secretion and elevated concentration of plasma insulin appeared to be the cause of decreased plasma NEFA concentration by partially inhibiting adipose tissue lipolysis. The reduction in prepartum NEFA concentration appeared to be the cause of decreased liver TG accumulation pre- and postpartum. Plasma NEFA and insulin concentrations, however, were not different post-calving, indicating prepartum elevations in insulin may only be beneficial in decreasing liver lipid accumulation prepartum. The overall effect of prepartum administration of propylene glycol was reduced total TG accumulation in the liver 1 and 21 d postpartum compared to cows that were not dosed with propylene glycol (64). Along with providing evidence of insulin resistance, Studer et al (64) work also indicates that insulin secretion may be suppressed during the transition period.

Adipocyte lipogenesis continues to be suppressed after the onset of lactation, the result of continued low levels of plasma insulin and diminished responsiveness of adipose tissue to insulin. The inability of adipose tissue to respond to insulin appears to be due to a postreceptor defect, because the ability of insulin to bind to its receptor is unaltered (68). This observation is consistent with results from another study (18) in which diminished responsiveness but not sensitivity to insulin in terms of whole-body glucose utilization was also observed in lactating goats. Faulkner and Pollock (20) reported that insulin was unable to suppress the release of NEFA and glycerol from adipose tissue, or amino nitrogen from

muscle, in ewes during early lactation. Vernon and Finley (68) also reported an insulin resistance in the hind limb of early lactation ewes; insulin administration was unable to stimulate glucose uptake by muscle. Therefore it appears that, early lactation is characterized by a moderate degree of insulin resistance in adipose tissue and muscle, resulting in the mobilization of NEFA from adipose tissue and AA from muscle, apparently to spare glucose for other productive functions.

Estradiol. Plasma concentrations of estradiol-17β in dairy cattle rise progressively during late gestation and peak 1 to 2 wk prior to parturition (7). The peak in estradiol has been thought to be responsible for the diminished feed intake by dairy cows in late gestation (21). Estradiol also appears to enhance NEFA mobilization from adipose tissue independent of any change in feed intake and energy balance (23). Grummer et al (23) observed that estrone, of placental origin, plays a role in the development of fatty liver during reduced feed intake by increasing hepatic fatty acid esterification, without a concurrent increase in VLDL-TG secretion. The specific effects of estradiol on hepatic lipid metabolism are unknown. However, it seems likely that estradiol influences hepatic lipid metabolism indirectly by causing a decrease in feed intake, resulting in elevated NEFA and liver TG concentrations.

Prolactin. Prolactin also may play a role in the modification of metabolic responses to the homeostatic signals in adipose tissue and other nonmammary tissues. Prolactin appears to inhibit the actions of insulin in adipose and mammary tissues of lactating rats (7). Prolactin also influences the partitioning of AA to the liver and extrahepatic tissues. Elevated prolactin concentrations in conjunction with increased insulin and growth hormone concentrations, as the result of venous arginine infusion prepartum, have been associated with a 10 % increase

in milk production postpartum (14). The authors suggested that the increase in prolactin acted synergistically with growth hormone to enhance mammogenesis and lactogenesis during late gestation to improve milk yield postpartum (14). The exact role of prolactin, however, in prepartum metabolism is unclear.

Growth Hormone. Growth hormone (GH) concentrations increase during late pregnancy, peak at parturition, and decline to moderately elevated levels in early lactation (7). The actions of GH in late gestation and early lactation appear to be mediated by altered tissue responses to other hormones such as insulin and the catecholamines (57). Therefore, GH appears to be the primary regulator of nutrient partitioning during the transition from pregnancy to lactation. This is consistent with the reported actions of GH. For example, Bauman and Vernon (5) and Sechen et al (58) reported that treatment of early lactation cows with GH decreased the rates of lipogenesis and increased the rates of lipolysis. The effects of GH on adipose tissue appear to be mediated via insulin responsiveness. The decrease in adipose tissue responsiveness to insulin is due to altered activity at the postreceptor level, because insulin receptor numbers and tyrosine kinase activity are unaltered in cows treated with GH (58). One possible mechanism is the reduction in the activities of lipogenic enzymes such as acetyl-CoA carboxylase in adipose tissue. Therefore, it would appear that the diminished rate of lipogenesis and increased rates of lipolysis during the periparturient period is due to elevated GH concentrations and low concentration of insulin (5, 7, 58).

Elevated GH concentrations during late gestation also may affect the lipolytic responses of adipose tissue to adrenergic agents, similar to the effect observed in the postpartum cow. Sechen et al (56) reported an enhanced lipolytic response to epinephrine

challenge in cows treated with GH during early lactation compared with control cows. This response was attributed to a reduction in epinephrine-stimulated rates of fatty acid esterification in adipocytes. The response to epinephrine appears to be at the postreceptor level, because there were no alterations in epinephrine clearance or sensitivity. The authors concluded that the postreceptor mechanism for increased lipolysis was via an increase in lipolytic enzyme activity. They suggested that the activity of hormone-sensitive lipase was increased in adipose tissue of GH treated cows (57).

Perhaps of greater importance to the transition cow is the influence of GH on liver metabolism. Lactating cows administered GH had elevated plasma NEFA concentration without a change in plasma ketone concentrations, suggesting that lipolysis was increased without a corresponding increase in hepatic fatty acid oxidation (47). Additionally, liver slices from dairy cows injected with GH increased hepatic gluconeogenesis in vitro (16, 37, 47). They attributed the increase to an increase (2.3 x controls) in glucose synthesized from propionate. Exogenous GH also increased acetate, lactate, and pyruvate conversion to glucose (37).

Of equal importance to the transition cow is GH influence on protein metabolism. Simmons et al (60) evaluated the effects of prepartum GH treatment on protein metabolism in multiparous cows injected with 0, 5, or 14 mg/d of GH daily for the last 46 d of gestation. They reported an increase in milk protein and solids-corrected milk yield with 14 mg/d of GH. Measures of protein metabolism, protein mass, and protein degradation were serum urea nitrogen, urine creatinine, and urine N^{τ}-methyl histidine- to -creatinine ratio, respectively. They reported that the 14 mg/d GH treatment prepartum increased AA uptake and nitrogen

retention from d 23 to d 1 prepartum as indicated by reduced serum urea nitrogen combined with increased urine creatinine concentrations compared to control cows. However, no difference in N^{τ}-methyl histidine- to -creatinine ratio during lactation indicated that protein degradation at parturition was not effected by prepartum treatment and increased lactational performance was related to greater protein reserves in GH treated cows (60).

Metabolic Adaptations during the Transition period

Numerous metabolic adaptations occur in the transition from pregnancy to lactation; these adaptations occur to accommodate the impending nutrient demands of milk synthesis. Furthermore, this period is often characterized by a dramatic decrease in feed intake commencing about 7 d before parturition (8, 25) despite increasing nutrient demands for the growing fetus and later for milk synthesis. Therefore, dietary nutrient concentrations and nutrient status during this period influence milk yield, milk component yields, and cow health.

The most notable change in nutrient needs is increased demand for gluconeogenic precursors. Bell (7) compared uterine uptake of glucose, AA, and fatty acids at 250 d of gestation and mammary uptake at 4 d postpartum in a mature 600 kg cow. He concluded that mammary requirements for glucose, AA, and fatty acids were approximately 2.7, 2.0, and 4.5-times those of the gravid uterus, respectively. Since intake does not parallel the increase in nutrient demand, a number of nonmammary metabolic adaptations occur to supply the lactating mammary gland with the necessary precursors. Liver, adipose tissue, and skeletal muscle are the tissues that are most directly involved with meeting the mammary demand for substrates.

Current recommendations (43) assume feed intake and nutrient requirements to

remain constant throughout the nonlactating period. However, Bertics et al (8) reported that when cows were fed to NRC recommendations prepartum, they experienced negative energy balance beginning 1 wk prepartum. Therefore, to meet both maternal and fetal energy needs, mobilization of body adipose tissue reserves occurs, as indicated by elevated prepartum plasma NEFA concentrations. The mobilization of fatty acids from adipose tissue during late gestation appears to be related to a reduction in de novo synthesis of fatty acids and TG. The activity of the two enzymes responsible for the decrease in fatty acid and TG synthesis, lipoprotein lipase and acetyl-CoA carboxylase, decrease during late pregnancy and remain low during early lactation (3).

It is at this time that fatty livers often result. Fatty liver occurs when the rate of hepatic TG synthesis exceeds the rate of TG hydrolysis plus TG export as VLDL (24). Along with the development of fatty liver, the rapid influx of plasma free fatty acids appears to lead to elevated rates of hepatic ketogenesis as indicated by increased plasma 3-hydroxy butyrate (24).

Current recommendations for feeding the periparturient cow focus on minimizing TG deposition in the liver and maximizing hepatic glycogen stores. Strategies to reduce the severity of fatty liver, and therefore ketosis, include decreasing fatty acid mobilization from adipose tissue, decreasing esterification of fatty acids in the liver, and increasing hepatic export of TG as VLDL (23). Regarding the last point, it has been suggested that the development of hepatic lipidosis during the periparturient period may be related to the biosynthesis and availability of VLDL constituents for packaging and export of VLDL (2). Bauchart (2) observed that apolipoprotein B mRNA concentrations were lower in the liver

of early lactation cows compared to dry cows or cows in midlactation, indicating that apolipoprotein B synthesis was limiting VLDL secretion. Additionally, Bauchart (2) indicated that inadequate availability of Met and Lys may limit apolipoprotein B synthesis. Durand et al (19) previously observed an increase in net hepatic output of VLDL when the livers of two early lactation cows were infused (via the mesenteric vein) with Met and Lys.

Curtis et al (17) reported that feeding diets containing greater protein concentration than that recommended by NRC (43) (12 % of diet DM rather than 8 % of diet DM) in the final 3 wk before calving decreased the incidence of retained placenta and primary ketosis. Grummer (25) explained that increasing prepartum dietary protein concentration above NRC (43) recommendations improves AA status in the prepartum cow and that the latter contributes to improvements in postpartum performance. Grummer (25) and Van Saun et al (65) suggested that underfeeding protein to the transition cow may deplete maternal reserves prior to lactation, leading to compromised lactation, health, and reproduction. Improving AA status of the prepartum cow may also influence endocrine physiology which can enhance lactation performance. For example, and as noted previously, venous infusion of arginine during late gestation increased blood prolactin, insulin, and growth hormone concentrations during late gestation and early lactation and increased milk yield by 10 % (14).

When cows were fed protein as RUP (6.0 versus 3.4 % of diet DM) during the last 3 wk of gestation, milk protein percentage increased, body condition improved, and incidence of postpartum metabolic disorders was reduced (65). The authors attributed the improvements in postpartum health and lactational performance to reduced mobilization of protein reserves. They reasoned, that during a period of declining intake, increasing the RUP

density of the diet allows nutrient intake to remain the same. Cows fed the higher protein diet were better able to support conceptus requirements than the cows fed the low RUP diet, resulting in minimal prepartal reserve depletion (65).

Increasing the postruminal supplies of specific essential AA (**EAA**) during late gestation has been shown to improve DMI and postpartum health in high producing cows (50). They reported that supplementing diets fed during the last 3 wk of gestation with ruminally protected Lys and Met reduced the incidence of metabolic disorders postpartum (50). One explanation for the improvement in postpartum health is that prepartum diets formulated according to NRC (43) recommendations for RUP and ruminally degradable protein (**RDP**) but without regard to AA may be limiting in Met and Lys, the two AA known to be the most limiting for lactating cows fed conventional diets (29, 53, 54). Furthermore, Met serves as a methyl donor in phospholipid synthesis and is required with Lys for apolipoprotein synthesis. Bauchart (2) suggested that when cows mobilize large amounts of fatty acids from adipose tissue to meet their energy needs, hepatic synthesis and export of fatty acids as TG-rich VLDL does not always increase, resulting in accumulation of lipid in the liver and moderate to severe hepatic lipidosis (see earlier discussions). Therefore, there is good evidence to suggest that increasing dietary RUP concentration of prepartum diets, or the supply of metabolizable protein in general, may improve postpartum milk production and cow health.

Early Lactation

The onset of lactation in high yielding dairy cows imposes dramatic alterations in nutrient demand. Unfortunately, the dramatic increase in nutrient demand is not accompanied

immediately by an increase in nutrient intake. The shortfalls in nutrient availability must therefore be made up by adaptations in adipose tissue, liver, and skeletal muscle metabolism. These alterations in metabolism occur to supply the lactating mammary gland with the substrates needed for lactose, protein, and fat synthesis.

Carbohydrate metabolism in the early postparturient cow is driven by the mammary requirement for glucose, because glucose is the precursor for lactose synthesis (37). Blood glucose for lactose synthesis arises from two sources; absorbed glucose and gluconeogenesis in liver and kidney (49). Dietary carbohydrates are fermented extensively in the rumen to volatile fatty acids, with propionate being the primary glucogenic volatile fatty acid. Reynolds et al (49) observed less than 15 % of blood glucose is derived from absorbed glucose and gluconeogenesis must provide the remaining glucose taken up by the mammary gland of lactating cows. When comparing mammary gland uptake of glucose with sources of glucose at 4 d postpartum, Bell (7) reported that glucose synthesized from propionate and AA provided for 65 % of mammary glucose uptake. Lactate of dietary and endogenous origin plus glycerol from adipose tissue accounted for another 15 to 20 % of the glucose requirement (7). Therefore, it is unlikely that hepatic metabolism of glucogenic precursors (propionate, glycerol, lactate, and AA) could meet mammary requirements for glucose, let alone the mandatory glucose needs of other tissues. Part of the shortfall in glucogenic substrate supply appears to be offset partially by reduced glucose uptake and oxidation in nonmammary peripheral tissues (4, 34).

The remaining deficit in glucogenic precursors appears to be made up by the mobilization of AA stored in skeletal muscle and other tissue proteins. Bauman and Currie

(3) estimated the labile protein reserve of the lactating cow to be approximately 15 to 25 % of total body protein. Although, the contribution of mobilized AA for mammary metabolism or hepatic gluconeogenesis up to peak lactation (42 to 56 DIM) appears to be small, it may be significant in the first 1 to 2 wk after parturition when the cow is in negative nitrogen balance (4). The most likely source of mobilized AA is skeletal muscle. Reid et al (48) measured a 25 % reduction in muscle fiber diameter in dairy cows immediately after calving and a decline in muscle protein: DNA ratio in early lactation ewes that were underfed protein during the transition period. This is consistent with the observation (12) of reduced muscle protein synthesis in early lactation goats that were in negative nitrogen balance. Boisclair et al (11) attributed the net release of AA from skeletal muscle when steers were in negative nitrogen balance to a suppression of protein synthesis rather than enhanced protein degradation. In addition to the release of AA from skeletal muscle, hepatic protein synthetic rates also appear to be augmented in early lactation (7). The increase in hepatic protein synthesis begins during late pregnancy and increases further at calving; however, the magnitude of this response and its regulation remains unclear (7, 60).

Along with adaptations in carbohydrate and protein metabolism, adaptations in lipid metabolism also occur in early lactation. Adipose tissue serves as an energy reserve (see earlier discussion) that can be called upon when energy intake is insufficient. Further, fatty acids mobilized from adipose tissue appear to spare glucose from use by extramammary tissues (24). Plasma NEFA also supply the mammary gland with a source of preformed fatty acids for milk TG synthesis, further sparing glucose for lactose synthesis.

Effect of Body Condition at Calving on Lactational Performance

The effect of prepartum body condition score (BCS) at calving on lactational performance has been studied widely (9, 10, 33, 44, 52). Generally, it is recommended that cows calve with a BCS of 3.5 (5-point scale; 1 = thin, 5 = obese). Body condition above 3.5 at calving has been linked to more severe negative energy balance, increased incidence of metabolic disorders, and poorer reproductive performance (44, 52). Pedron et al (44) reported that cows with BCS of 4.0 or greater at calving have reduced DMI, increased plasma NEFA concentrations, and lower plasma insulin concentration than cows calving with a BCS of 3.5 or less. The relationship between prepartum body condition and milk yield is also influenced by early lactation diet. Cows that calve with more body condition (BCS > 3.5) respond to increased dietary concentrations of RUP, whereas thinner cows (BCS < 3.5) respond to increased dietary energy density (52). It is expected that a cow will lose body condition during early lactation; peak milk yield and loss of body condition occur simultaneously. Ruegg et al (52) reported greater daily milk yields from cows that lost > 0.75 condition points by 60 to 80 days in milk as compared to cows that lost < 0.75 points. This indicates that loss of body condition in early lactation is essential to maximal lactational performance. To evaluate the interaction of BCS at calving and postpartum dietary RUP, Seymour and Polan (59) fed cows either to gain body condition (high energy diet) or to maintain body condition (low energy diet) during the dry period; after calving, thin and heavy cows were assigned to either a high RUP or a low RUP diet. They reported that heavy cows fed the high RUP postpartum diet produced more milk, consumed less dry matter, and lost more body condition than thin cows, regardless of the postpartum diet fed (59). Seymour and

Polan (59) hypothesized that there is an interaction between dietary RUP and body condition loss in early lactation and that increased metabolizable protein will enhance the efficiency of use of adipose tissue reserves for milk synthesis.

Effect of Increasing Dietary RUP

Protein also may limiting for early lactational performance. Many workers have investigated the response to additional dietary protein, specifically the response to increases in RUP (32, 35, 36, 38, 59, 65). Generally, increasing the supply of protein that reaches the small intestine increases yields of milk and milk protein (35) but, results are variable. Responses to added RUP may be inconsistent because increases in RUP may have resulted in a shortage of RDP, fermentable carbohydrate, or both, which would decrease synthesis of microbial CP (MCP) and the contribution of MCP to supplies of metabolizable protein (35, 63). Other reasons for no response to increased dietary RUP include a less favorable profile of AA in metabolizable protein, decreased intestinal digestibility of over-treated or over-processed supplemental protein, or other limiting dietary factors (e.g. energy) (35, 63). Keery et al (35) reported that when solvent-extracted soybean meal was replaced by heat-treated soybean meal in diets of early lactation cows, milk and milk protein yields increased as well as the efficiency of CP utilization for milk protein production. Cows receiving the heat-treated soybean meal consumed more RUP and that more metabolizable protein was available as compared to the soybean meal treatment. However, cows receiving the heat-treated soybean meal diet lost more weight; the weight loss could be accounted for by the additional 44.8 kg of 4 % fat-corrected milk that was produced during the first 8 wk of lactation. The authors proposed that increasing the concentration of RUP in the diets of cows in negative

energy balance may increase the supply of AA available for absorption, resulting in improved efficiency in use of mobilized nutrients (35).

Metcalf et al (41) proposed that milk protein output can be increased by increasing dietary protein concentration, suggesting that milk protein synthesis is driven partially by substrate supply. However, in an experiment with 4 chronically catheterized cows fed diets containing increasing amounts of heat-treated soybeans to provide 113, 154, or 201 g of CP/kg of DM; milk protein output did not increase proportionally with increasing arterial concentrations of EAA (41). Milk protein output also did not increase linearly with EAA uptake by the mammary gland. Part of the discrepancy between arterial supply and uptake of EAA by the mammary gland and milk protein output appeared to be related to an unchanged supply of NEAA to the mammary gland. The authors concluded that EAA supply was not the primary limitation to milk protein synthesis and that a proportion of milk AA may have to be supplied in a bound form for milk protein synthesis. They also concluded that a high degree of interconversion of AA must occur within the mammary gland.

The precise mechanisms that regulate milk quantity and milk composition remain unclear. Guinard and Rulquin (27) and Guinard et al(28) infused incremental amounts of casein (0, 177, 352, or 762 g/d) into the duodenum of high producing cows fed corn-based diets to meet 90, 100, 110, and 130 % of the protein requirements of each cow. Yields of milk and milk protein increased linearly with infusion of casein. Increased yields of milk protein with casein infusion were associated with increased arterial concentration and mammary gland extraction of total AA (essential plus nonessential). Mammary gland extraction of blood NEFA, 3-hydroxy butyrate, and glucose also increased with duodenal

casein infusion, despite no changes in plasma concentrations (27). Several workers (29, 30, 53, 54, 55) have reported increased milk and milk protein yields as well as increases in milk protein content when cows were infused duodenally with Lys, Met, or both Lys and Met. Lactational responses to improved Lys and Met nutrition indicate that supplies of Lys and Met were limiting nutrients. Collectively, these studies indicate that milk and milk protein synthesis are limited by AA, particularly Lys and Met, and energy substrate.

Production responses of dairy cows to improved Lys and Met nutrition include variable increases in content and yield of milk protein, milk production, and feed intake. As summarized by Guinard and Rulquin (29) and Schwab (56), experiments confirm that responses to postruminal Lys and Met supplementation are greater when basal levels of Lys and Met in RUP are low rather than high, when intake of RUP is high rather than low, when cows are in early lactation rather than mid or late lactation, and in high producing cows rather than low producing cows. Furthermore, it is noteworthy that (1) content of milk protein is more sensitive to duodenal concentrations of Lys and Met than is milk yield, (2) increasing the amounts of Lys and Met in metabolizable protein increases generally only the casein fraction of milk protein and not the whey and NPN fractions, (3) increasing the amounts of Lys and Met in metabolizable protein generally increases the content of milk protein more than what would be expected by increasing diet CP, (4) the relationship between duodenal concentrations of Lys and Met and content and yield of milk protein is described by typical curves of diminishing increments (27, 28, 61), and (5) increases in milk yield to supplemental Lys and Met generally are limited to cows in early lactation when the need for absorbable AA relative to absorbable energy is greatest.

In an attempt to determine the benefits of improving Lys and Met nutrition during late gestation and early lactation, Socha et al (61) assigned eighty-four multiparous cows to a randomized block experiment with a 2 x 3 factorial arrangement of treatments. Two weeks prior to parturition, cows received the same basal diet (15.9 % CP) with either no rumen-stable AA, 10.5 g of rumen-stable Met (supplied as 15 g/d of Smartamine M™), or 10.2 g of rumen-stable Met plus 16.0 g of rumen-stable Lys (supplied as 6 g/d of Smartamine M™ and 40 g/d Smartamine ML™). From parturition through d 105 postpartum, cows continued to receive the AA treatments but were switched to diets balanced for 16.0 and 18.5 % CP. The AA treatments were designed to raise the estimated levels of Lys and Met in duodenal digesta from 14.0 and 4.1 % to 14.8 and 4.6 % of total EAA, respectively. Yields of milk and milk components were similar for cows receiving no rumen-stable AA and cows receiving rumen-stable Met. In contrast, supplementing with Lys plus Met increased DMI and yields of energy-corrected milk and milk protein, and tended to increase yield of milk, fat-corrected milk, and content of milk protein. Responses to Lys plus Met tended to be greatest during the first 11 wk of lactation and when fed in conjunction with the 18.5 % CP diet. Milk yields increased 9, 12, 7, and 6 % during wk 1-3, 4-6, 7-9, and 10-12 of lactation, respectively; plasma glucose decreased and there was an apparent, but nonsignificant increase in plasma NEFA concentrations with no apparent effect on liver lipid content. The decrease in plasma glucose was attributed to greater utilization of glucose for lactose synthesis. The trend toward greater NEFA concentration was considered to reflect increased adipose tissue mobilization. The authors also noted an apparent improvement in liver lipid export with Lys and Met supplementation, since supplemented cows had greater NEFA concentrations but

liver lipid concentration was unaffected.

Milk Urea Nitrogen as an Indicator of Protein Adequacy

The need to assess dietary protein adequacy and efficiency of nitrogen utilization has resulted in the development of techniques to evaluate milk urea nitrogen (**MUN**). Urea in the blood originates as ammonia, released either from cellular deamination of AA or from microbial deamination of AA in the rumen as the result of microbial degradation of RDP. Rumen microbes use fermentable carbohydrates and ammonia to synthesize AA, and when the concentration of ammonia exceeds the need for AA synthesis in the rumen, ammonia is absorbed via the rumen wall and converted to urea by the liver (40). Milk urea arises primarily from passive transfer of urea from blood; therefore, MUN and plasma urea nitrogen (**PUN**) are highly correlated (31, 40, 51). Urea concentration in blood and milk reflect the percentage of dietary CP, the amount of RDP in total CP, the amount of fermentable organic matter in the diet, and postruminal protein metabolism (36, 51). The efficiency of use of RDP for microbial protein synthesis is dependent on the amount and source of fermentable carbohydrate in the diet. Generally, increasing the intake of fermentable carbohydrate increases MCP synthesis and decreases ruminal ammonia concentration (15). Therefore, as the imbalance of RDP to fermentable carbohydrate increases, PUN and MUN increase, indicating inefficient use of RDP (15, 36, 51). Elevated MUN and PUN concentrations also reflect an imbalance in the AA profile of metabolizable protein reaching the small intestine, and improving AA balance will reduce MUN and PUN concentrations (40). Linn and Olson (40) have summarized the effects of dietary carbohydrate, RDP, RUP, and intestinal AA balance on milk CP percentage and MUN (Table 1) in early lactation cows (0 to 45 DIM).

Knowledge of MUN concentrations in conjunction with milk CP concentrations can be useful in assessing adequacy of carbohydrates and protein feeding.

The objectives of the current experiment were to determine the effects of and interaction between, feeding additional RUP and rumen-stable Lys and Met during the last 3 wk of gestation on DMI, BW changes, body condition, and subsequent lactational performance and cow health, and to determine the benefits of feeding rumen-stable Lys and Met during the first 10 wk of lactation on the same parameters.

Table 1. Interpretation of milk urea nitrogen (MUN) in early lactation.[1]

Milk crude protein, %	Low < 12	Moderate 12 to 18	High > 18
	MUN, mg/dl		
< 3.0	Protein or RDP deficiency	RDP adequate, low RUP and fermentable CHO	RDP in excess of fermentable CHO; imbalance of AA
3.0 to 3.2	Low RDP compared to energy	RDP/RUP/AA balanced	Excess RDP; AA and energy in balance
> 3.2	Low RDP with balanced AA and excess energy	Adequate RDP, RUP, and AA with excess energy	Excess RDP and energy; AA in balance

[1] Adapted from Linn and Olson (40), where early lactation is 0 to 45 DIM.

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CHAPTER II

DRY MATTER INTAKE, PASSAGE OF NITROGEN FRACTIONS, AND AMINO ACIDS TO THE SMALL INTESTINE, AND BLOOD METABOLITES DURING THE PERIPARTURIENT PERIOD OF HOLSTEIN COWS

Abstract

Starting 14 d prior to expected calving, 10 multiparous, Holstein cows were fed a TMR that contained (% of DM) 33.2 corn silage, 22.1 haycrop silage, 29.9 ground shelled corn, 11.6 soybean meal, 0.7 blood meal, 0.7 tallow, and 1.9 mineral mix. After calving, all cows were fed a TMR that contained (% of DM) 20.6 corn silage, 20.6 haycrop silage, 35.9 ground shelled corn, 17.0 soybean meal, 1.4 blood meal, 1.4 tallow, and 4.0 mineral mix until 12 DIM. Feed intake was measured daily; milk production was measured daily during the 12 d postpartum. Blood samples were collected daily from the tail vein 3 h post-feeding. Five cows previously fitted with ruminal and duodenal cannulas were used for collection of ruminal and duodenal digesta. Duodenal digesta was collected from cannulated cows every 4 h. Samples were composited within cow over consecutive 48-h periods (e.g., d -1 and -2, d 1 and 2, etc.) relative to calving. Ruminal digesta was collected from cannulated cows twice weekly (6 h post-feeding) for recovery of mixed bacteria. Mean daily DMI values were 15.1 and 16.5 kg for the pre- and postpartum periods and mean daily milk production for the first 12 DIM was 35.7 kg. Mean plasma glucose concentration was 70.1 mg/dl prepartum, 80 mg/dl on the day of calving, and 63 mg/dl postpartum; corresponding mean NEFA

concentrations in plasma were 126 prepartum, 493, and 478 mEq/L . Flow of DM to the duodenum averaged 11.5 kg/d prepartum and 11.4 kg/d postpartum; flow was lowest the first 2 d postpartum (8.6 kg/d). Corresponding flows of N fractions to the duodenum over the duration of the experiment were very similar; the mean contribution of microbial N to total N was 56%. In a similar fashion, Lys and Met, as percentage of total essential amino acids in duodenal digesta also were similar throughout the experiment; mean contributions of Lys and Met were 15 and 5% prepartum and 16 and 5% postpartum, respectively.

(**Key words:** periparturient period, glucose, nonesterifed fatty acids, lysine, and methionine)

(**Abbreviation key**: **AA** = amino acids, **ADF** = acid detergent fiber, **CP** = crude protein, **DIM** = days in milk, **DM** = dry matter, **DMI** = dry matter intake, **EAA** = essential amino acids, **Lys** = lysine, **Met** = methionine, **NEAA** = nonessential amino acids, **NEFA** = nonesterified fatty acids, **NDF** = neutral detergent fiber, **NSC** = non-structural carbohydrates, **OM** = organic matter, **RDP** = ruminally degradable protein, **RUP** = ruminally undegradable protein)

Introduction

Protein requirements of dairy cows during late pregnancy and the earliest stages of lactation are not well defined. Assuming diets of normal energy (1.25 Mcal of net energy per kg of DM) and neutral detergent fiber (**NDF**; 35 % of DM) contents, the recommended crude protein (**CP**) content of diet DM for pregnant dry cows during the last 2 months of gestation is 12 % (17). No recommendations are provided for distribution of ruminally degradable (**RDP**) and ruminally undegradable (**RUP**) protein in total CP. Moreover, the NRC (17) assumes a constant daily net protein requirement of 1.136 g/kg $BW^{0.70}$ for the conceptus after 210 d of gestation and a constant efficiency of converting absorbed protein to net protein of

0.50.

Recent studies indicate that following NRC (17) guidelines for diet CP may not always provide for adequate metabolizable or absorbable protein during late gestation. For example, Van Saun and Sniffen (24) reported that mature, prepartum Holstein cows fed additional amounts of RUP (total CP at 120 and 150% of NRC, 1989) had a reduced incidence of clinical ketosis and reduced days open compared with cows fed a similar diet containing less protein. Holtenius and Hjort (13) reported that prepartum cows fed a higher protein diet had lower serum nonesterified fatty acid (**NEFA**) concentrations, NEFA to cholesterol ratios, and fatty liver scores. Results of these studies are consistent with the conclusions of an earlier literature summary (9) which indicated that feeding diet concentrations of protein greater than recommended by NRC (17) beginning 3 wk prepartum reduced incidences of uncomplicated ketosis and retained placenta after calving. Feeding diets with additional RUP during late gestation have also improved body condition at parturition (13, 24); similar observations have been noted in beef cattle (15) and sheep (13). These studies indicate that the amino acid (**AA**) requirements of the late gestation dry cow are higher than traditionally thought and that insufficient protein during late gestation may accelerate depletion of labile protein reserves, thereby increasing postpartum metabolic disorders and directly or indirectly, impairing reproductive performance. Bell et al (5) provided convincing evidence that the metabolizable protein requirements for conceptus growth during late gestation is at least two to three times greater than that accounted for by the NRC (17) recommendation. Moreover, the NRC (17) guideline for diet CP concentration

does not account for the reduction in feed intake as parturition approaches.

Positive postpartum responses to prepartum RUP supplementation suggest that opportunities exist for improving the profile of AA passing to the small intestine during late gestation. Lysine and Met appear generally to be the most limiting EAA for lactating dairy cows (19). Regression analysis of measurements of AA passage to the duodenum in lactating dairy cows indicated that increasing the concentrations of RUP or total CP in diet DM will decrease the contributions of Lys and Met to total EAA in duodenal digesta (20). This is an expected observation as total EAA in most feed proteins have lower concentrations of Lys, Met, or both as compared to ruminally synthesized microbial protein (7). For that reason, milk and milk protein responses of lactating dairy cows to supplemental Lys and Met, provided either by intestinal infusion or by feeding in ruminally protected form, are greater when intake of CP is higher rather than lower (1, 18, 20).

There are no published studies of measured flows of N fractions and AA to the small intestine of cows during the periparturient period. Fundamental to a better understanding of the AA nutrition of cows during this period of transition is knowledge of any potential effects that changes in DMI, gut fill, and resulting ruminal digestibility may have on predicting nutrient passage to the small intestine. The primary objectives of this experiment were to measure changes in ruminal digestibility and in passage of N fractions and AA to the small intestine from late gestation to early lactation and to determine if changes occurred in the profile of EAA presented to the duodenum that could not be explained by diet composition. The Cornell Net Carbohydrate and Protein System with associated Amino Acid Submodel

(CNCPS) (3) is the most dynamic of the models described to date and was used to predict passage of AA to the small intestine. Another objective was to obtain preliminary data on changes in plasma glucose and NEFA concentrations during the periparturient period when cows were fed a nutrient-dense prepartum diet.

Materials and Methods

Cows and Management

Five multiparous Holstein cows previously fitted surgically with ruminal and closed-T duodenal cannulas and five intact multiparous Holstein cows were used in the experiment. Duodenal cannulas were placed 10 to 15 cm distal of the pylorus. The experiment was initiated 12 d before expected calving and terminated at 12 DIM. Cows were assigned to the experiment over a 42-d period in December and January. Cows were housed individually on rubber mats in tie stalls in an insulated, naturally ventilated barn.

Diet and Feeding

The diets fed before and after calving are presented in Table 1. The prepartum diet was offered a minimum of 14 d before expected calving and was formulated to exceed NRC (17) requirements for nonlactating, late gestation cows. The postpartum diet was formulated to meet or exceed slightly NRC (17) recommendations for lactating cows. Both diets were fed as a TMR three times daily at 1530, 2030, and 0500 h and orts were weighed once daily at 1400 h. Cows were fed to ensure ad libitum intake, but refusals were kept to a minimum of 5 to 10 % of intake.

Measurements and Sample Collection

Feed ingredients were sampled before the experiment, then daily throughout the experiment. Daily feed samples were composited weekly. Ort samples were obtained weekly beginning 14 d before calving. Feed composites and ort samples were dried at 55°C for 24 h, and ground to pass through a 1-mm screen before sending to Northeast Dairy Herd Improvement Corporation (Ithaca, NY) for analysis of CP, neutral detergent fiber (**NDF**), acid detergent fiber (**ADF**), ether extract, Ca, P, K, Mg, and S. Milk weights were recorded daily for the 12-d period after calving.

The five cannulated cows were used to recover mixed ruminal bacteria for N and purine analysis, to measure ruminal digestibility of organic matter (**OM**), NDF, ADF, and N, and to measure passage of N fractions and AA to the small intestine. Chromium oxide was used as an unabsorbable digesta flow marker for intestinal flow measurements. Three grams of chromium oxide were encapsulated in filter paper and placed in the ruminal mat at 0400, 1000, 1600, and 2000 h via the ruminal cannula. Administration of chromium began 10 d before duodenal sampling commenced and continued throughout the experiment. Duodenal samples (500 ml) were collected every 4 h every day from 14 d prior to parturition through 12 DIM. At the end of the experiment for each cow, digesta samples were thawed, composited over 2-d (48-h) periods on either side of the parturition date (d -1 and -2, d 1 and 2, etc.), and composites homogenized in their entirety by using a large commercial blender. One sample of each composite was strained through a double layer of cheesecloth, centrifuged, and the supernatant fluid assayed for ammonia. A larger sample was freeze-dried

and allowed to air equilibrate for 12 h and ground through a 1-mm screen. Homogenized composites (about 6-L each) were poured continuously between two containers to ensure no separation of liquid and particulate fractions. Digesta samples were analyzed for DM, OM, N, ADF, NDF, AA and chromium.

Samples of ruminal digesta were collected twice weekly from the reticulum near the reticulo-omasal orifice 6 h after feeding. Immediately after collection, ruminal digesta was homogenized in a 3.8 L commercial blender (Waring Products Division, New Hartford, CT) for 30s on low speed to remove loosely-associated microorganisms from particulate matter. The homogenate was strained through one layer of 59-μm Dacron mesh, and the fluid was retained. Mixed fluid and particle-associated bacteria were isolated from ruminal digesta by centrifuging at 500 x *g* for 20 min to remove protozoa followed by centrifugation of the supernatant fluid at 20, 000 x *g* for 20 min. Cells were washed with 0.85 % saline, centrifuged, and washed again with distilled water.

Blood samples were obtained daily from all ten cows from 14 d before expected calving through 12 DIM. Sampling was by venipuncture of the coccygeal vein at approximately 3 h after the 0500-h feeding. Blood was collected into 10-ml evacuated tubes containing sodium heparin and 4% sodium fluoride. Tubes were placed immediately in an ice bath and were centrifuged within 45 min at 3500 x *g* for 20 min at 5°C. Plasma was stored at -20°C until analyzed for glucose and NEFA.

Analytical Procedures

Dry matter content of duodenal digesta, feed composite, and ort samples were

determined by drying at 60° C under 760 mm of vacuum for 24 h . Organic matter content of feeds and duodenal digesta was determined by ashing at 500° C for 16 h in a muffle furnace. Chromium content of duodenal samples was determined using atomic absorption spectrophotometry (Thermo Jarrell Ash, Model Smith-HIEFTJE12, Franklin, MA) according to the procedure of Williams et al(27). Neutral detergent fiber content of feed and duodenal samples was determined using the procedure of Van Soest et al (25) and N content was determined by the macro-Kjeldahl procedure and CP calculated by N x 6.25 (2). Content of ADF in feed, ort, and duodenal samples was determined according to AOAC procedures 973.18 (2). Non-structural carbohydrate content of feeds and duodenal samples was calculated by difference based on chemical analysis [100 % -(CP % + NDF % + EE % + ash %)].

Concentrations of AA in feeds, duodenal digesta, and bacteria were determined using the procedure outlined by Cunningham et al (8). Briefly, 150 mg of sample were hydrolyzed with 15 ml of 6 *N* HCL in the presence of 65 mg of dithiodipropionic acid and .75 ml of 10 % phenol in an anaerobic chamber. Culture tubes were removed from the chamber and incubated at 105° C for 22 h. Samples were centrifuged (12,500 x g for 20 min at 5° C), filtered (Metricel Membrane Filter, pore size .45 μm; Gelman Sciences, Ann Arbor, MI), and a 1-ml aliquot was dried by vacuum in the presence of NaOH. Individual AA, including Cys, were determined using a Beckman 7300 AA analyzer (Beckman Instruments Inc., Palo Alto, CA). Duodenal flows of individual AA were calculated by multiplying the AA concentrations in digesta (milligrams per gram DM) by the calculated DM flow.

Ruminal and duodenal digesta fluids were analyzed for ammonia using a gas-ammonium electrode (Orion model 407A meter with 95-12 electrode; Orion Research, Inc., Boston, MA) following the procedure of Schwab et al (19). Bacterial isolates were dried in a convection oven at 60°C for 18 h; samples were taken to complete dryness by drying in a vacuum oven at 60°C for an additional 18 to 24 h before analysis for N and purine content. Bacteria were analyzed for N and AA according to the described procedures. The purine content of bacteria was determined using the procedure of Zinn and Owens (29) as modified by Ushida et al (22). Briefly, bacterial samples (0.2 g) were mixed with 2.5 ml of 70 % perchloric acid, placed in a 95° C water bath for 10 min, vortexed, and the tubes returned to the bath for another 1 h. Thereafter, 17.5 ml of 0.0285 M ammonium phosphate buffer were added and after for 15 min at 95° C, the hydrolysate was filtered (G8 glass fiber filters; Gelman Sciences, Ann Arbor, MI) and 9 ml of 0.2 M ammonium phosphate buffer and 0.5 ml of 0.4 M silver nitrate were added to the filtered hydrolysate. The mixture was centrifuged (20,000 x g for 10 min) and the supernatant fluid was removed by aspiration. The pellet was washed with 10 ml of distilled water, adjusted to pH 2.0 with sulfuric acid, recentrifuged, and the fluid supernatant removed by aspiration. Ten ml of 0.5 *N* HCL were added to the pellet; samples were then vortexed, incubated in a 95° C water bath for 1 h, centrifuged, filtered (Whatman no. 451 filters; Gelman Sciences, Ann Arbor, MI), and absorbance read spectrophotometrically at 260 nm. Yeast RNA was used as a standard.

Duplicate plasma samples were assayed for glucose using a colorimetric-enzymatic assay (Glucose Trinder, Sigma Diagnostics, St. Louis, MO). A separate aliquot was assayed

in triplicate for NEFA (Wako Chemical, Neuss Germany).

Results and Discussion

The chemical compositions of the offered and consumed diets are in Table 2. The AA composition of feedstuffs is in Table 3. The primary objective of the experiment was to measure changes in ruminal digestibility and passage of N fractions and AA to the small intestine during the transition from late gestation to early lactation. To minimize the effects of diet differences on these measurements, the prepartum diet was formulated with the same ingredients and similar nutrient profile to the postpartum diet but without being too dissimilar from what might be considered and acceptable prepartum diet. Evaluation of the diets with the CNCPS indicates that both diets contained adequate RDP and that neither total N or peptides were limiting for microbial protein synthesis (Table 3). Concentrations of Lys and Met in total EAA (excluding Trp) passing to the duodenum were estimated to be 16.0 and 5.2 % for the prepartum diet and 15.3 and 4. 7 % for the postpartum diet, respectively.

Dry matter intake decreased as parturition approached and on the day of calving was 15 % less than the average intake between 12 and 6 d prepartum (Figure 1). Grum et al (11) reported a similar percentage decrease in DMI on the day of calving (estimated from intakes the day before and the day after calving) as compared to 2 wk before calving. In that study, cows were fed either a control, low energy diet (69.8 % chopped oat hay, 26.0 % ground shelled corn, and 3.0 % soybean meal; 12.7 % CP) or a high grain, high energy diet (51.0 % oat hay, 42.6 % corn, and 4.6 % soybean meal; 13.2 % CP) from dry-off until 7 d before expected parturition date. At that time, cows were adapted to a lactation diet by feeding an

adaptation diet of 66.7 % oat hay and 33.3 % of the lactation diet. However, Bertics et. al. (6) reported a sharp decline in DMI beginning 1 wk prepartum with intake being approximately 30% less on the day of calving as compared to 7 to 21 d before calving. Van Saun et al (24) reported a 38 % decrease in DMI during the last 10 d of gestation when first-lactation Holstein cows were fed either a control (12.4 % CP and 3.4 % RUP; DM basis) or a high RUP diet (15.3 % CP and 6.0 % RUP).

Passage of DM to the small intestine tended to parallel DMI (Figure 1.). Dry matter apparently digested in the rumen as a percentage of DMI averaged 20.8 % for the prepartum period and 31.9 % for the postpartum period.

The N and purine contents of ruminal bacteria are presented in Figure 2. The N content remained similar during the periparturient period. However, the total purine content of bacteria appeared to be influenced by DMI with concentrations being highest at the beginning and at the end of the periparturient period and the lowest at parturition. A decrease in the purine to N ratio of ruminal bacteria around the time of parturition when feed intake, and presumably ruminal turnover of digesta, is depressed is consistent with decreased bacterial growth. Ribonucleic acid makes up a large part of bacterial cells and ribonucleic acid to N ratios decrease rapidly in response to slower growth rates in pure cultures of ruminal bacteria (4) and non-ruminal bacteria (10). The use of "experimental means" for marker and N content of bacterial cells for quantifying microbial protein in duodenal digesta requires that the ratio of microbial marker to microbial N remains constant during the course of the experiment. Our results indicate that measurements of microbial N passage to the small

intestine during the periparturient period requires isolation of ruminal bacteria on the same day as collections of abomasal or duodenal digesta for measurements of N and purines. The experimental means for concentrations of N and purines in bacterial OM (8.1 and 8.4 %, respectively) in this study are within the ranges reported by others (7).

The relationships between DMI, OM and fiber digestion, and passage of N fractions to the small intestine have been evaluated in lactating cows (7). In brief, as feed intake increases, the amount of OM fermented in the rumen increases, resulting in increased microbial protein synthesis and passage to the small intestine. The effect of increased OM fermentation appears to result from both a greater intake of fermentable carbohydrate and an improvement in efficiency of microbial protein synthesis. The increase in efficiency of microbial protein synthesis is likely due to a reduction in recycling of energy and N within the rumen which decreases microbial maintenance requirements and provides more nutrients for microbial growth. Feeding more RUP increases the quantity of dietary protein that passes to the small intestine and because of reduced intake of either RDP or fermentable carbohydrate, would be expected to decrease the quantity of microbial protein that is synthesized in the rumen.

Ruminal digestibility of OM, fiber fractions, and N appeared not to be influenced markedly by changes in diet or DMI during the periparturient period (Figure 3) in this experiment, and ruminal pH was 6.0 or greater throughout the periparturient period (Figure 4), indicating that changes in diet and DMI did not adversely influence ruminal fermentation. Organic matter digestibility appeared to be slightly greater than NDF digestibility before

calving but similar to or lower than NDF digestibility after calving (Figure 3). The apparent decrease in digestibility of NSC after parturition may be related to increased passage of both fluids and solids with increased intake (7). Clark et al (7) reported that increases in intake in early lactation increase fluid and solid passage to the small intestine, partially due to increased OM intake and partially because of increased OM fermentation. Ruminal ammonia concentrations were higher after calving than before calving (Figure 4), reflecting the higher concentration of RDP in the postpartum diet (11.2 % of DM) than in the prepartum diet (9.8 % of DM).

Changes in N passage to the small intestine, as expected, followed that of N intake (Figure 5). Duodenal flows of AA also followed a pattern similar to that of DMI (Table 4). Flows of bacterial-N and non-ammonia non-microbial N fractions to the small intestine generally paralleled each other throughout the periparturient period (Figure 6). As a result of the similar proportional flows to the small intestine of bacterial N and non-ammonia non-microbial N, the profile of EAA in the total protein passing to the small intestine remained similar throughout the periparturient period (Figure 7). Most of the small changes in measured EAA profiles of digesta protein between the prepartum and postpartum periods (Figure 7) are explained by the CNCPS (3) (Table 5). For example the Cornell model predicts Lys to be 15.4 % of EAA (excluding Trp) when the prepartum diet is fed and 15.3 % of EAA when the postpartum diet is fed (Table 5); mean values for measured contributions of Lys to total EAA for the two respective periods of time were 15.3 and 15.3%. These results indicate that knowledge of diet composition is sufficient for predicting the profile of

EAA presented to the duodenum during the periparturient period, at least when a nutrient-dense prepartum diet is fed and that any potential effects that changes in DMI, gut fill, and resulting changes in ruminal fermentation may have on passage of AA to the duodenum is limited to absolute flows of AA (Table 4) with no apparent effects on EAA profiles (Table 5).

Plasma NEFA concentrations (Figure 8) began increasing 2 d prior to parturition, peaked 2 d postpartum, and declined steadily thereafter. Increased NEFA prior to parturition indicates adipose tissue mobilization in response to decreased DMI (6) and indicates negative energy balance prior to the onset of lactation (6). The changes in plasma NEFA observed in this experiment agree with changes reported by others (6, 11, 26). Despite greater energy and protein concentrations of the prepartum diets, NEFA concentration during the last 12 d of gestation and first 12 d of lactation indicate that cows mobilized body tissue to support nutrient demands during a period of insufficient intake. Bertics et al (6), Vazquez-Anon et al (26), and Grum et al (11) attributed the increase in NEFA 2 d prior to calving to a change in hormones such as the catecholamines and glucocorticoids. Catecholamine and glucocorticoid concentrations increase as parturition approaches, in response to diminished intake and the stress of parturition (26).

Glucose concentrations (Figure 8) began increasing 4 d before calving and peak concentrations occurred on the day of calving. The glucose response in this study has been observed previously (6, 11, 26) and was attributed to an increase in hepatic gluconeogenesis in response to diminished intake and increased stress. Grum et al (11) observed a trend in

greater glucose concentration during the periparturient period when cows were fed above the NRC (17) requirement for protein and energy. They attributed the greater glucose concentration to an increase in gluconeogenic substrate, namely AA (11). Plasma glucose concentrations decrease after calving because of increased uptake by the lactating mammary gland for lactose synthesis.

Conclusions

Feeding a high energy-dense (NE_L = 1.6 Mcal/kg of DM), higher CP containing pre-partum diet with the same ingredients as the postpartum diets stabilized rumen fermentation during the periparturient period and minimized the decrease in feed intake that accompanies parturition. Similar proportional flows of bacterial N and non-ammonia non-microbial N to the small intestine throughout the periparturient period resulted in a stable profile of AA in duodenal digesta. The profile of EAA presented to the duodenum could be explained fully by diet composition. Despite the increase in nutrient density of the prepartum diet, plasma concentrations of NEFA indicated that cows still experienced a period of negative energy balance.

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TABLE 1. Ingredient composition of offered diets[1].

Ingredient	Prepartum	Postpartum
	(% of DM)	
Corn silage[2]	33.2	20.6
Haycrop silage[3]	22.1	20.6
Ground shelled corn	29.9	35.0
Soybean meal, solvent extracted 44% CP	11.6	17.0
Blood meal	.7	1.4
Fat[4]	.7	1.4
Minerals and vitamins[5]	1.9	4.0
Anionic salt[6]	.01	...

[1] Percentages are average values for the approximate 56-d period when each of the diets were fed.

[2] Treated at ensiling with .3 % urea. Contained 31.8 % DM, and as a percent of DM, contained 43.5 % NDF, 25.3 % ADF, and 8.5 % CP.

[3] Contained 31.0 % DM, and as a percent of DM, contained 59.1 % NDF, 38.5 % ADF, and 15.5 % CP.

[4] Alifet™ (Alifet USA, Inc., Cincinnati, OH).

[5] Contained: 15.5 % Ca, 5.6 % P, 4.6 % Mg, 1.5 % K, 2.1 % S, 0.27 % Zn, 0.13 % Mn, 0.23 % Cu, 0.0025 % I, 0.0041 % Co, 0.0013 % Se, 29513 IU/kg of vitamin A, 9443 IU/kg of vitamin D, and 94 IU/kg of vitamin E.

[6] Contained: 0.15 % Ca, 0.84 % P, 6.86 % Mg, 0.65 % K, 7.82 % S, 0.16 % Zn, 0.11 % Mn, 0.22 % Cu, 0.0022 % I, 0.0022 % Co, 0.0043 % Se, 50050 IU/kg of vitamin A, 12551 IU/kg of vitamin D, and 335 IU/kg of vitamin E.

TABLE 2. Chemical composition and measures of nutritive value of offered and consumed[1] diets.

	Prepartum		Postpartum	
Item	Offered	Consumed	Offered	Consumed
Chemical composition				
DM, %	44.5	44.5	50.9	50.9
CP, % of DM	15.8	15.8	18.5	18.6
RUP[2], % of DM	6.0	6.0	7.4	7.4
RDP[2], % of DM	9.8	9.8	11.1	11.2
RUP, % of CP	38.0	38.0	40.1	39.8
NSC [3], % of DM	40.6	40.6	41.5	43.3
NDF, % of DM	30.6	30.8	24.9	24.3
ADF, % of DM	18.1	18.3	14.6	14.3
EE[4], % of DM	4.2	4.8	4.7	4.8
Ca, % of DM	0.5	0.5	0.7	0.7
P, % of DM	0.3	0.3	0.3	0.3
Mg, % of DM	0.3	0.3	0.4	0.4
K, % of DM	1.3	1.3	1.3	1.3
S, % of DM	0.3	0.3	0.3	0.3
Nutritive value				
NE_L[5], Mcal/kg of DM	1.7	1.6	1.7	1.7
Rumen N balance[6], g/d	+5.0 (+ 1.8%)		+75.0 (+25%)	
Rumen peptide balance[6], g/d	+4.0 (+ 2.2%)		+59.0 (+34%)	
Rumen pH[6]	6.3		6.1	
Duodenal digesta Lys[6], % of EAA[3]	15.4 (15.4)[7]		14.8 (15.3)[7]	
Duodenal digesta Met[6], % of EAA[3]	5.0 (5.0)[7]		4.6 (4.7)[7]	

[1] The chemical composition of consumed diets was calculated by dividing the difference between the quantities of offered and refused nutrients by DMI.

[2]Calculated from NRC (17).

[3] Non-structural carbohydrates = 100% - (% NDF + % CP + % EE + % ash).

[4] **EE** = ether extract, **EAA** = essential AA. Essential AA includes Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val. Values in parentheses for Lys and Met do not include Trp as part of EAA.

[5] Calculated from NRC (17) equations.

[6]Calculated using the Cornell Net Carbohydrate and Protein System (3).

[7] Values in parentheses for Lys and Met do not include Trp as part of EAA.

TABLE 3. Amino acid composition of feed ingredients.

Amino Acid	Corn silage	Haycrop silage	Ground shelled corn	Solvent soybean meal	Blood meal[1]
Ala	.78	1.08	.73	2.10	6.64
Asp	.47	1.22	.71	5.52	8.38
Cys	.15	.26	.21	.60	.02
Glu	.95	1.18	1.77	8.66	7.71
Gly	.32	.68	.38	2.01	3.74
Ser	.28	.52	.45	2.30	4.38
Tyr	.21	.41	.37	1.59	2.61
Arg	.18	.44	.45	3.48	3.53
His	.13	.23	.26	1.23	4.52
Ile	.26	.54	.30	1.96	.90
Leu	.74	1.07	1.17	3.59	9.97
Lys	.21	.61	.30	2.95	7.35
Met	.10	.16	.17	.48	1.41
Phe	.28	.61	.46	2.27	5.61
Thr	.26	.55	.35	1.87	4.21
Val	.39	.80	.46	2.20	7.02
	(% of essential AA[2])				
Arg	7.1	8.7	11.4	17.4	7.9
His	4.9	4.6	6.6	6.2	10.1
Ile	10.0	10.8	7.6	9.8	2.0
Leu	28.9	21.4	29.9	17.9	22.4
Lys	8.3	12.2	7.7	14.7	16.5
Met	4.0	3.3	4.2	2.4	3.2
Phe	11.1	12.1	11.8	11.3	12.6
Thr	10.2	11.0	9.0	9.0	9.5
Val	15.4	15.9	11.8	11.0	15.8

[1] Ring-dried blood meal.
[2] Excludes tryptophan; tryptophan was not measured.

TABLE 4. Duodenal flows of amino acids in cows fed the prepartum and postpartum diets.

	Prepartum diet (2-d periods)							Postpartum (2-d periods)					
AA	-12	-10	-8	-6	-4	-2	Calving	2	4	6	8	10	12
	(g/d)												
Essential													
Arg	110	143	122	128	104	87	94	100	115	117	127	152	172
His	55	76	61	64	54	44	48	51	62	62	64	76	87
Ile	107	137	119	125	101	85	91	96	110	120	120	144	162
Leu	214	269	228	237	196	162	176	189	196	235	232	279	318
Lys	149	207	176	183	151	126	134	142	159	170	177	212	237
Met	53	68	58	62	51	45	47	50	55	61	59	72	79
Phe	127	165	123	151	114	92	94	96	118	123	123	142	164
Thr	104	139	128	138	110	94	100	106	124	126	126	153	172
Val	86	114	109	147	98	82	88	94	117	117	116	140	158
Total	1006	1317	1124	1235	979	817	870	923	1055	1132	1144	1372	1549
Nonessential													
Ala	161	211	181	182	151	127	136	145	172	175	174	212	240
Asp	237	315	272	278	229	195	209	222	262	268	273	327	368
Cys	36	45	38	40	34	29	31	33	39	39	37	47	51
Glu	325	407	354	361	296	258	276	294	345	356	355	424	485
Gly	132	173	156	173	138	122	129	135	155	163	162	197	228
Pro	119	146	128	131	108	93	99	106	122	125	125	151	173
Ser	117	151	132	134	111	95	101	108	126	131	130	156	177
Tyr	102	129	111	117	96	82	86	91	104	115	112	135	150
Total	1227	1577	1371	1415	1163	1001	1068	1135	1325	1373	1368	1649	1873
EAA/total AA	.45	.46	.45	.46	.46	.45	.45	.45	.44	.45	.46	.45	.45

TABLE 5. A comparison of predicted[1] and measured profiles of essential AA (EAA) in total protein passing to the small intestine of cows during the prepartum and postpartum periods.

Amino acid	Prepartum		Postpartum	
	Predicted	Measured	Predicted	Measured
Arg	13.0	10.7	13.2	10.9
His	5.5	5.5	5.8	5.6
Ile	11.0	10.4	10.6	10.5
Leu	17.0	20.2	17.4	20.2
Lys	15.4	15.3	15.3	15.3
Met	5.0	5.2	4.7	5.2
Phe	10.8	11.9	10.9	10.7
Thr	10.2	11.0	10.0	11.2
Val	12.1	9.8	12.1	10.3

[1] Calculated using the Cornell Net Carbohydrate and Protein System (3).

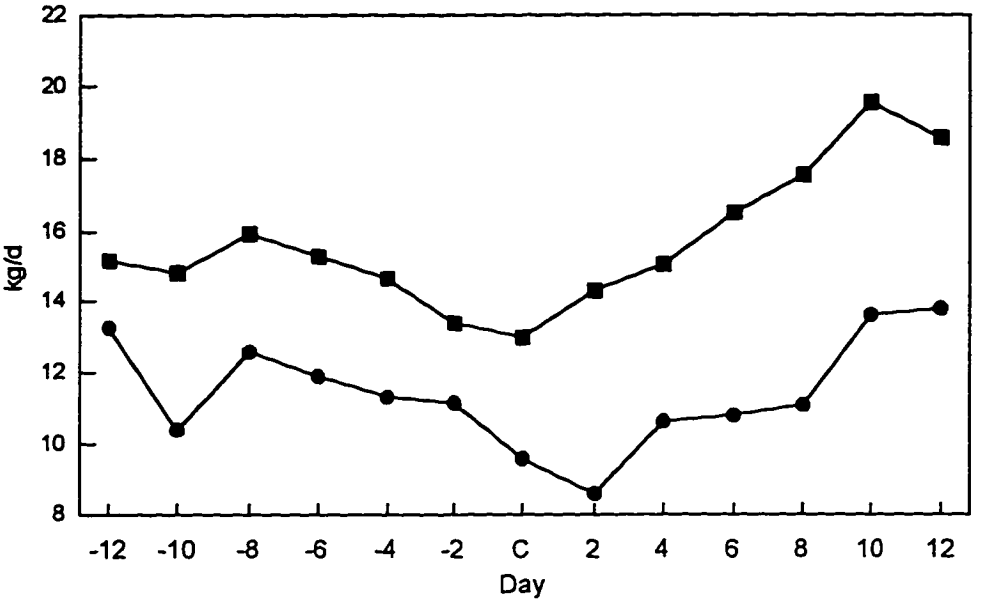

Figure 1. Intake of (■) and passage to the duodenum (●) of DM in cows between 12 d prepartum through 12 d postpartum. Pooled standard deviations are 2.96 and 4.5 kg/d for DMI and DM flows, respectively.

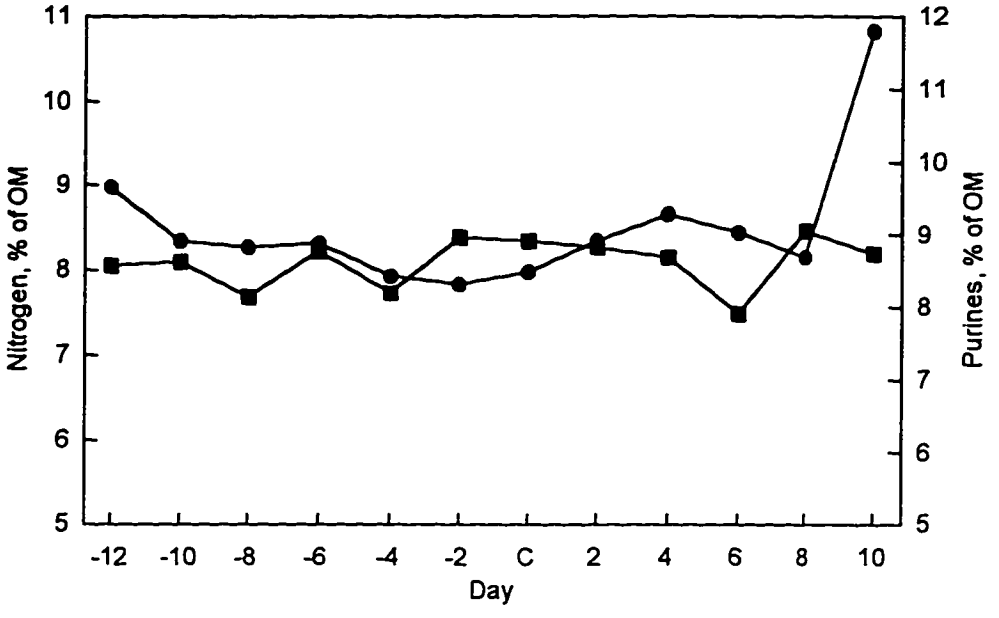

Figure 2. Total N (■) and purine (●) contents of ruminal bacteria of cows from 12 d prepartum through 12 d postpartum. Pooled standard deviations are 0.3 and 2.6 % of OM for N and purines, respectively.

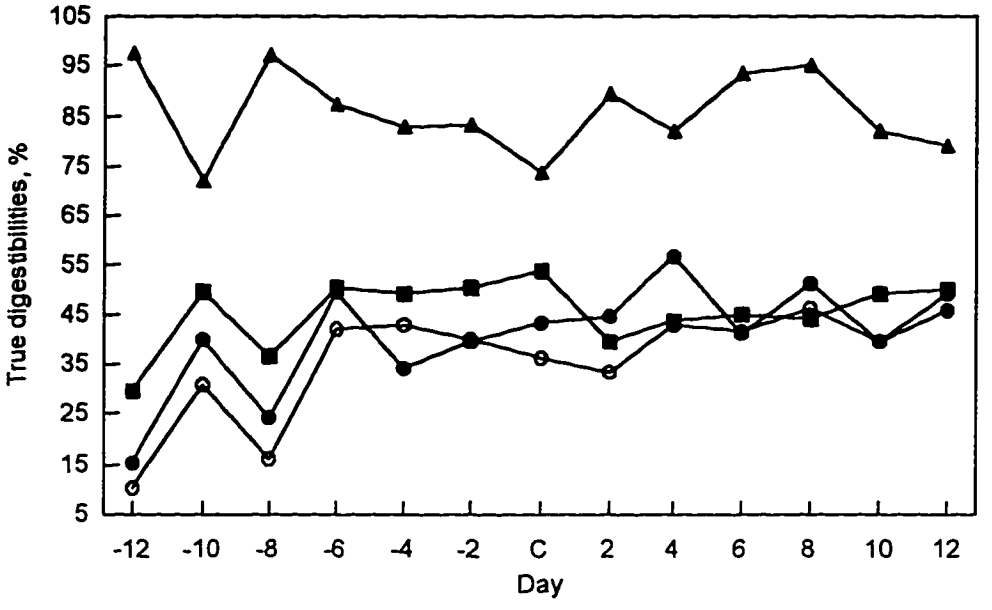

Figure 3. True digestibilities of OM (■), NDF (●), ADF (○), and N (▲) as measured in cows from 12 d prepartum through 12 d postpartum. Pooled standard deviations are 15.1, 12.5, 12.6, and 22.2 % for OM, NDF, ADF, and N, respectively.

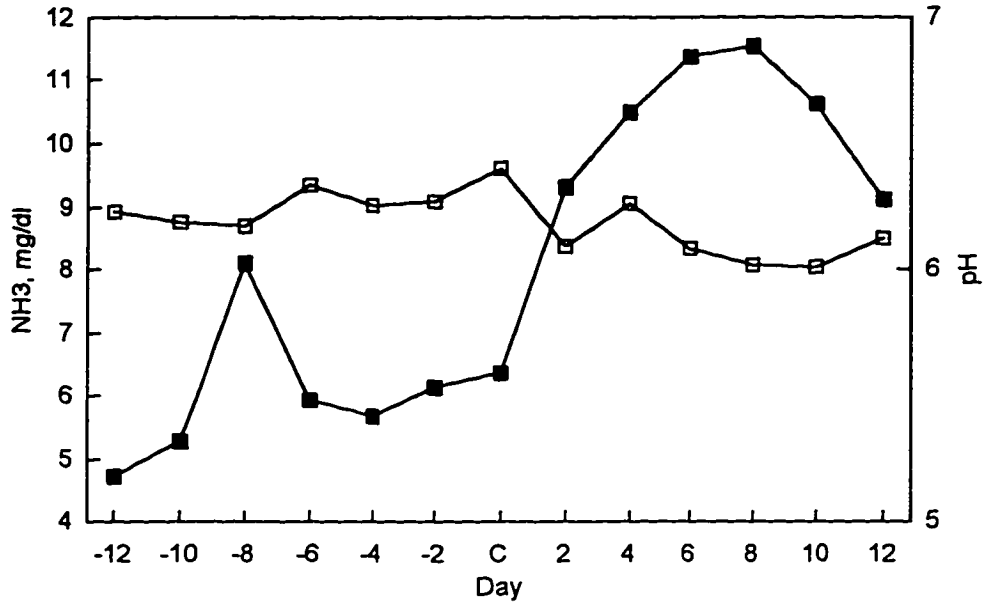

Figure 4. Ammonia concentrations (■) and pH (□) of ruminal contents of cows from 12 d prepartum through 12 d postpartum. Pooled standard deviations are 4.4 mg/dl and 0.18 for ammonia and pH, respectively.

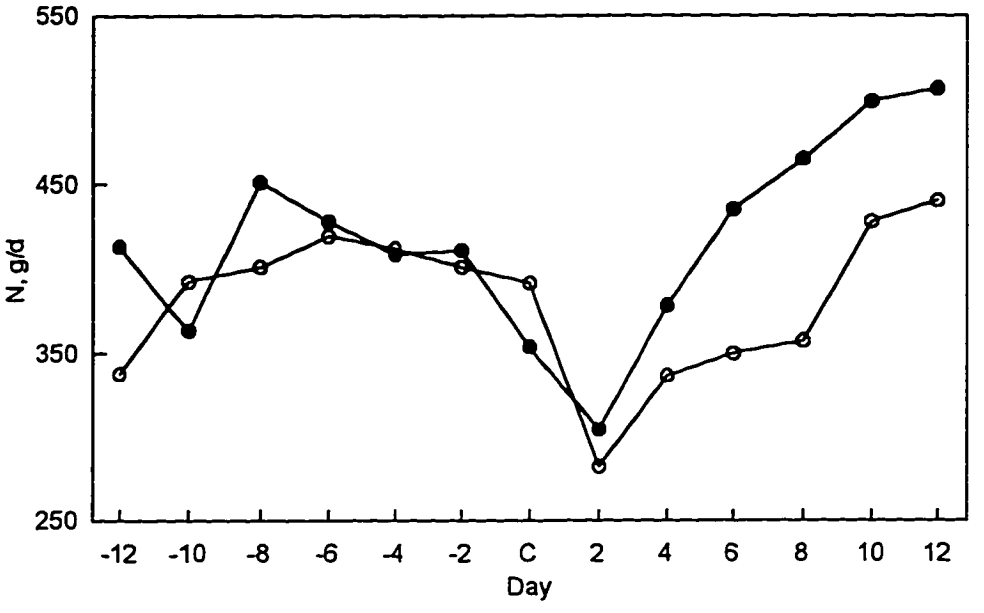

Figure 5. Intake of N (○) and N passage to the small intestine (●) of cows from 12 d prepartum through 12 d postpartum. Pooled standard deviations are 90.6 and 57.7 g/d for N intake and N passage, respectively.

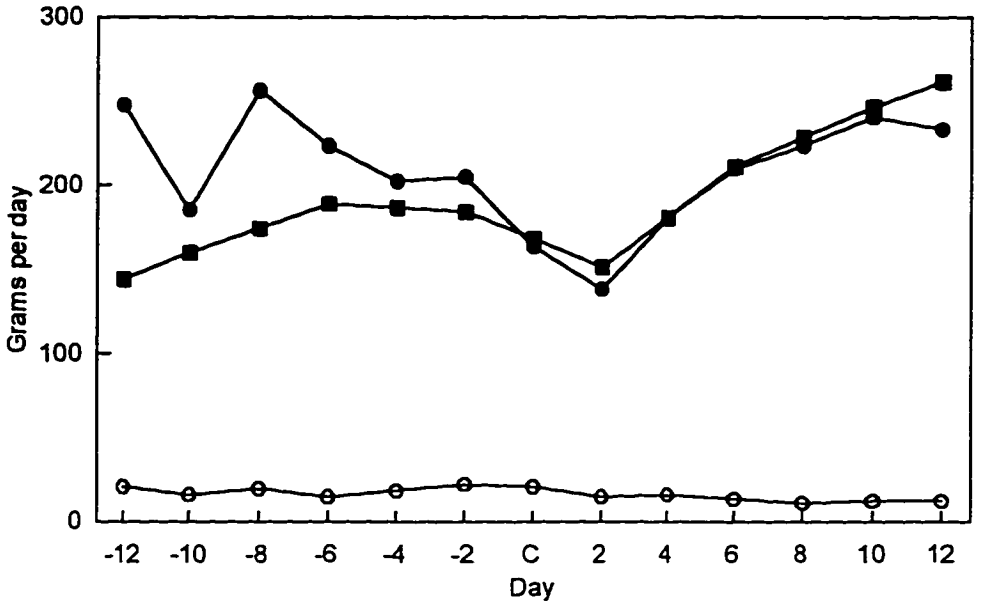

Figure 6. Passage of bacterial N (■), non-ammonia non-microbial N (●), and ammonia N (○) to the small intestine of cows from 12 d prepartum through 12 d postpartum. Pooled standard deviations are 36.0, 34.6, and 0.76 g/d for bacterial N, non-ammonia non-microbial N, and ammonia N, respectively.

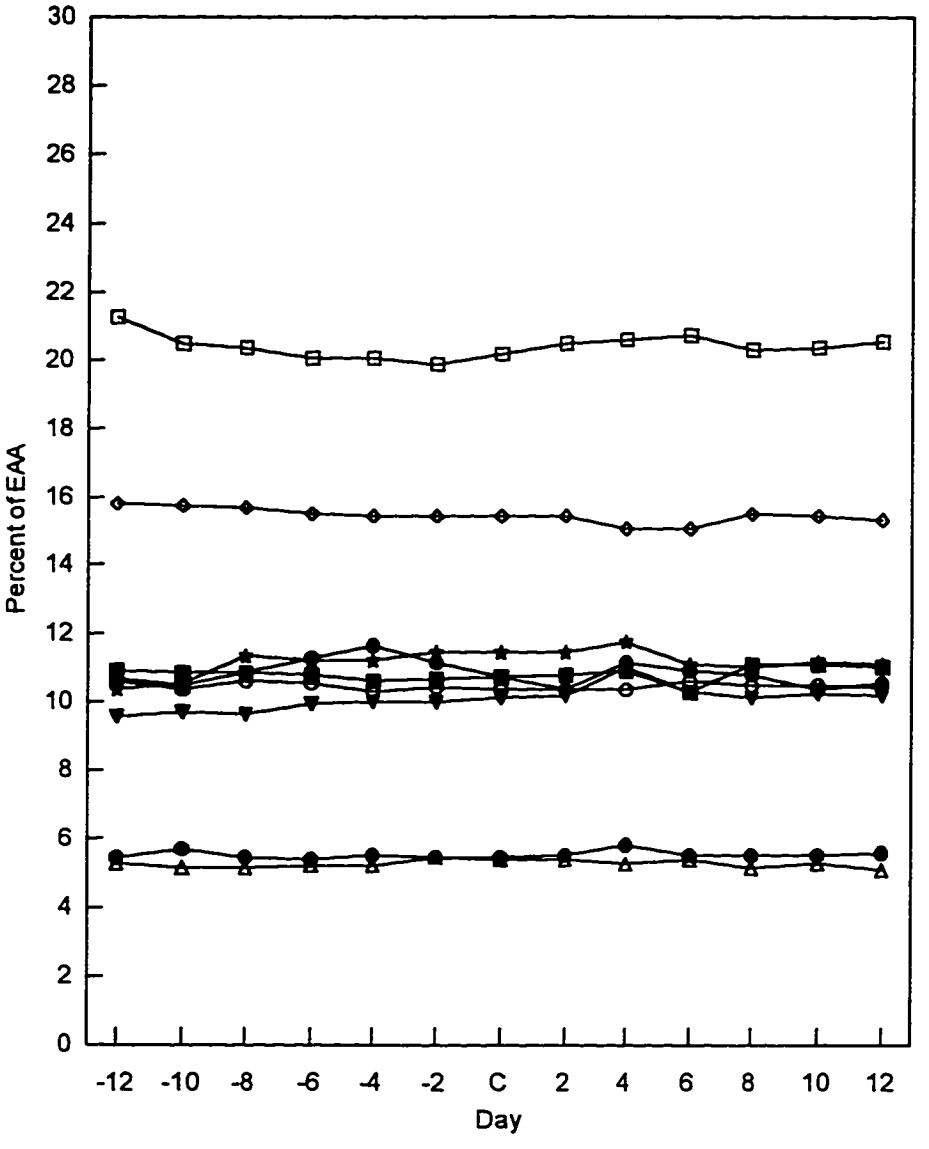

Figure 7. Contributions of Arg (▲), His (●), Ile (■), Leu (□), Lys (◊), Met (△), Phe (○), Thr (★), and Val (▼) to total essential AA (EAA) in duodenal digesta of cows from 12 d prepartum through 12 d postpartum. Pooled standard deviations are .44, .27, .46, .26, .72, .25, .34, .50, and .70 % for Arg, His, Ile, Leu, Lys, Met, Phe, Thr, and Val respectively.

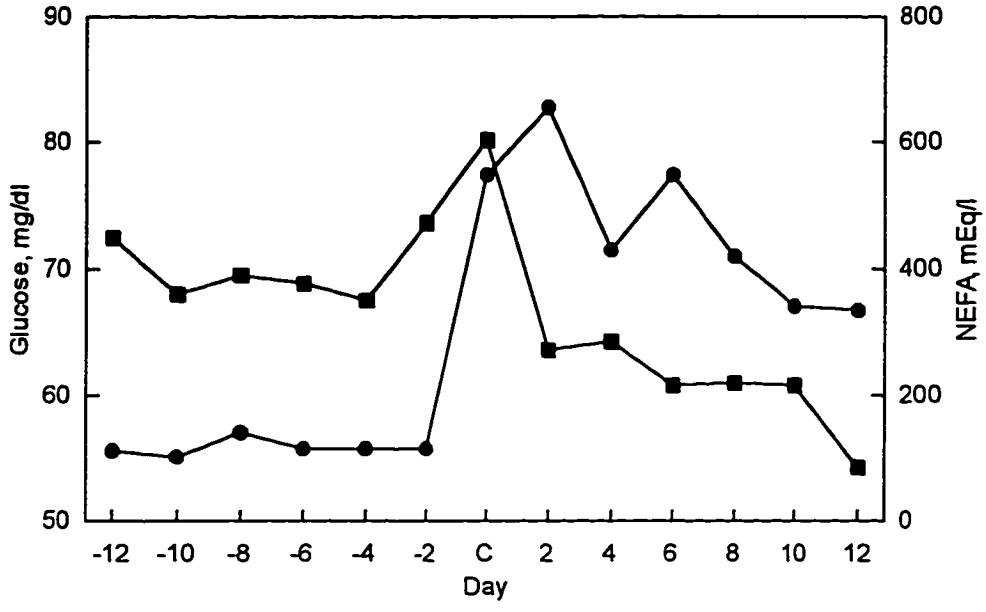

Figure 8. Glucose (■) and NEFA (●) concentrations in plasma of cows from 12 d prepartum through 12 d postpartum. Pooled standard deviations are 12.3 mg/dl and 186.4 mEq/l for glucose and NEFA, respectively.

CHAPTER III

OPTIMIZING LYSINE AND METHIONINE NUTRITION DURING THE PERIPARTURIENT AND POSTPARTURIENT PERIODS. I. MILK AND MILK COMPOSITION RESPONSES

Abstract

Seventy-two multiparous cows were blocked by expected calving date and assigned to a 2 x 2 factorial arrangement of dietary treatments beginning 21 d before expected calving. The prepartum diets were (% of DM) low RUP(4.9 % RUP and 13.8 % CP), low RUP plus 5.2 g/d of rumen-stable Lys and 4.5 g/d of rumen-stable Met, high RUP (6.6 % RUP and 15.6 % CP), and high RUP plus 4.6 g/d of rumen-stable Lys and 8.3 g/d of rumen-stable Met. Each block contained eight cows and two cows per block received each of the prepartum dietary treatments. On the day of calving, one cow from each pair received a basal lactation diet (7.3 % RUP and 18.1 % CP) and the other cow received the basal diet plus 12.9 g/d of rumen-stable Lys and 19.5 g/d of rumen-stable Met. Diets were corn-based and all supplemental protein was provided by soybean products. Cows remained on treatments until 70 d postpartum. Cows fed the high RUP diets lost less weight after calving that the cows fed the low RUP prepartum diets. Dry matter intake during the first 10 wk of lactation was greatest for cows that received the high RUP prepartum diets and the Lys and Met supplemented lactation diet and lowest for cows fed the high RUP prepartum diets and the

unsupplemented lactation diet. Inclusion of Lys and Met in the lactation diet decreased milk yields (44.5 vs. 46.6 kg/d). Milk fat and milk urea N concentrations, and yields of milk fat, crude protein, and true protein were not affected by dietary treatments. However, true protein concentrations were greater for cows fed the high RUP prepartum diets than cows fed the low RUP diets (2.96 vs. 2.79%) and greater for cows fed the Lys and Met supplemented lactation diet that cows fed the unsupplemented diet (2.93 vs 2.77%). Increases in milk protein with postpartum Lys and Met were greatest when high RUP prepartum diets were fed (+.23 percentage unit) vs. when low RUP prepartum diets were fed (+.10 percentage unit) and when AA were not included in the prepartum diets (+.23 percentage unit) vs. when they were included in the prepartum diets (+.10 percentage unit). Concentration of RUP and concentrations of Lys and Met in RUP of prepartum diets both appear to affect the milk protein content response of early lactation cows to supplemental Lys and Met.

(**Key words:** Lysine, methionine, periparturient, and postparturient)

Abbreviation key: AA= amino acids, **BCS**= body condition score, **BW**= body weight, **CP**= crude protein, **DMI** = dry matter intake, **Lys** = lysine, **Met** = methionine, **RDP**= ruminally degradable protein, **RUP**= ruminally undegradable protein

Introduction

A fundamental goal of late gestation nutrition is to allow the dairy cow to make the transition from pregnancy to lactation without complications and with feed intakes commensurate with optimum rates of tissue mobilization. To that end, the focus of many recent experiments has been to acquire a better understanding of organic nutrient metabolism

during the transition from late gestation to early lactation and to fine-tune late gestation diets to minimize postpartum metabolic disorders while maximizing subsequent lactational performance (3, 9, 39).

Adequate intakes of ruminally degradable (**RDP**) and ruminally undegradable (**RUP**) protein are required for an uneventful transition from pregnancy to high levels of milk production. It generally is accepted that the current NRC (23) crude protein (**CP**) recommendations underestimate both maternal and fetal needs during the last 3 wk of gestation (3, 9, 39). Greater dry matter intake (**DMI**), both before and after calving, increased yields of milk and milk protein, and a reduced incidence of postpartum metabolic disorders have been reported when the CP content of the prepartum cow diet is increased above NRC (23) recommendations, particularly when the increase in CP is RUP (9, 24, 39, 42). Increasing prepartum diet CP appears to increase both prepartum and postpartum DMI and milk yield by preventing prepartal accumulation of lipid in the liver (2, 4, 9). This is consistent with the observation that when higher CP and energy diets are fed during late gestation, mobilization of adipose (and protein) reserves before parturition is reduced (4, 8, 39).

Variable responses to increasing RUP in postpartum diets have been reported (10, 11, 17, 18, 42). In some studies increasing dietary RUP did not increase milk or milk protein yields (11, 17, 18, 20, 42). Lack of a response to increased dietary RUP has been attributed to decreased dietary RDP and thus lower yields of microbial protein (6, 10, 18), decreased intestinal digestibility of total diet RUP (1, 10), or a less optimum profile of absorbed amino acids (**AA**) (26, 35).

Lysine and Met have been implicated most often as the two most limiting AA for milk protein synthesis (28). That Lys and Met are generally the two most limiting AA appears to be a result primarily of the fact that most feed proteins, and thus the RUP fractions, have lower amounts of Lys and Met, relative to total essential AA (EAA) than what the cow requires (26, 35). The greatest responses to diet supplementation of ruminally protected forms of Lys and Met have occurred in early lactation (1, 36). Armentano et al (1) reported greater milk protein concentration and yield in cows supplemented with 5.6 g/d Met and 16.6 g/d Lys, during early and midlactation. Cows were fed corn-based diets with solvent soybean meal, cottonseed, and corn gluten meal as the protein supplements. Increases in milk protein were greater when cows were in early lactation compared to midlactation and the authors attributed the increase in milk protein concentration and yield to a greater supply of Lys and Met for milk protein synthesis. Further, greater response in milk protein to Lys and Met supplementation during early lactation was attributed to greater deficiency of Lys and Met during early lactation compared to midlactation (1). Supplementing diets with intestinally available, rumen-stable forms of Lys and Met during the first 9 wk of lactation increased DMI, yield of energy-corrected milk and milk CP yield (36). Cows were fed corn-based diets with solvent soybean meal and blood meal as the protein sources. Socha et al (36) also implied that response to Lys and Met was greatest during early lactation due to a greater deficiency of these two AA and that cows at this stage of lactation are more responsive to improvements in intestinal AA balance. Lysine and Met also may be the two most limiting AA during late gestation. Feeding ruminally protected Lys and Met during the final 3 wk of gestation reduced the incidence of metabolic disorders at parturition and increased milk and

milk protein yield in the first 10 wk of lactation (24).

The primary objective of the current experiment was to determine the effects of, and interaction between, feeding greater amounts of RUP and supplemental Lys and Met during the last 3 wk of gestation. The second objective was to determine the benefits of supplemental Lys and Met during the first 10 wk of lactation when all supplemental protein in a corn-based diet was provided by soybean products. Dry matter intake, changes in body weight (BW) and body condition, and subsequent lactational performance, and cow health were examined as performance criteria.

Materials and Methods

Experimental Design and Treatments

All procedures related to animal care were conducted with the approval of the University of New Hampshire Institutional Animal Care and Use Committee. Seventy-two multiparous cows were blocked by expected calving date and assigned to a 2 x 2 factorial arrangement of treatments consisting of either 4.9 or 6.6 % RUP and either 13.8 or 15.6 % CP beginning 21 d before expected calving. The four treatments, differing in RUP and total CP and differing in the absence or presence of ruminally protected Lys and Met, were 1) low RUP no Lys and Met; 2) low RUP plus 5.2 g/d of Lys and 4.5 g/d of Met; 3) high RUP diet no Lys and Met; and 4) high RUP plus 4.6 g/d of Lys and 8.3 g/d of Met. Lysine and Met were provided in a rumen-stable form (Smartamine™ M and Smartamine™ ML, Rhône-Pôulenc Animal Nutrition, Atlanta GA) and were fed in amounts to achieve intestinal concentrations of 15.5 and 5.5% of total essential AA for Lys and Met, respectively as

estimated by the regression equations of Socha (35). Each block contained eight cows and two cows per block received each of the prepartum diets. On the day of calving, one cow from each pair within a block received the basal lactation diet and the other cow received the basal lactation diet plus 12.9 g/d of rumen-stable Lys and 19.5 g/d of rumen-stable Met (Table 1). Cows remained on the lactation diet until 70 DIM. Three cows were removed from the experiment; two due to environmental mastitis and one because of an abomasal ulcer.

Feeding and Management of Cows

Diets (Table 1) were fed as a TMR and were prepared by weighing each ingredient and mixing in a drum type mixer (Data Ranger; American Calan, Inc. Northwood, NH). Orts were collected and weighed daily at 1000 h. Amounts of lignosulfonate and heat-treated soybean meal (SoyPass®, Ligno Tech USA, Inc., Overland Park, KS) and solvent-extracted soybean meal were adjusted to achieve RUP and RDP concentrations in the diet DM of 4.9 and 8.9 % and 6.6 and 9.0 % for the low and high RUP prepartum diets; the basal lactation diet was formulated to contain 7.3 % RUP and 10.8 % RDP. Cows were fed 50 % of the total daily allotment at 1400 h, 30 % at 2000 h, and 20 % at 0400 h. Rumen-stable AA were topdressed at the time of feeding, with 50 % at 1400 h, 30 % at 2000 h, and 20 % at 0400h.

Measurements, Collection, and Analysis of Samples

Prior to the iniation of the experiment and every week thereafter, forages and orts were sampled for DM determination, dried to 88 % DM, ground to pass through a 1-mm screen, and composited by block of the experiment. Forage and orts composites were analyzed for CP, neutral detergent fiber (NDF), acid detergent fiber (ADF), Ca, P, K, Mg, and S (NEDHIC, Ithaca, NY). Concentrate feeds were analyzed every 3 mo for CP, NDF,

ADF, Ca, P, K, Mg, and S (NEDHIC, Ithaca, NY).

Milk weights were recorded three times daily, and milk samples were taken from three consecutive milkings on two nonconsecutive days each week. Milk samples were preserved with 2-bromo-2-nitropropane-1,3 diol and composited by individual milk weight before analysis for fat, CP, true protein and milk urea nitrogen (NEDHIC, Ithaca, NY). Body condition scores (BCS) were obtained weekly, using a 5 point scale (1= thin, 5= obese) with quarter-point divisions, by three individuals and averaged for each week starting 21 d before expected calving date. Cows were weighed weekly beginning 21 d before expected calving.

Statistical Analysis and Calculations

Repeated measurements for the prepartum and postpartum data (DMI, BW, and BCS) were reduced to total experimental means. Milk, milk composition, milk component yields, BW, and BCS after calving represent the least square means of the three-way interaction of prepartum diet RUP, prepartum diet AA, and lactation diet. The GLM procedure of SAS (27) was used to analyze the data, and means were considered different at $P< 0.10$.

The model for analysis was a randomized block design with a 2 x 2 factorial arrangement of treatments. Total first lactation milk yield and initial BW (21 d prior to expected calving) were used as covariates for DMI, milk yield, BW, and BCS. Milk fat percentage from each cow's first lactation and initial BW were covariates for milk fat; milk CP, milk true protein, and milk urea nitrogen were adjusted using each cows first lactation milk CP percentage and initial BW. First lactation data were used as covariates because they are good predictors of subsequent lactational performance and control variation among individuals (5). Change in body condition and BW were calculated as the difference between

the average BCS or BW at wk 7 of lactation and the average BCS or BW before calving (-2 wk and -1 wk).

Results

Diets

The mean chemical composition of the consumed basal diets and predicted concentrations of Lys and Met in duodenal digesta without Lys and Met supplementation are presented in Table 2. The chemical composition of consumed diets differed little from the mean chemical composition of formulated diets (data not shown). The additional RUP in the high RUP prepartum diets replaced NDF; concentrations of NSC remained constant at 42.4 % and RDP remained similar at 8.9 to 9.0 % of DM.

Body Weight and Body Condition

Body weight and BCS and their respective changes for the first 70 DIM are shown in Table 3. Body weights were not affected by treatments. However, prepartum RUP concentrations affected BW change during the first 10 wk of lactation. Cows fed the low RUP prepartum diets lost more weight after calving than the cows fed the high RUP prepartum diets (123 vs. 102 kg). The additional weight loss of the cows fed the low RUP prepartum diets in the first 10 wk of lactation appeared to be associated with greater mobilization of body fat as changes in BCS tended to be greater in cows fed the high RUP prepartum diets (-.81 vs -.65). Cows fed the low RUP prepartum diets followed by the basal lactation diet maintained greater body condition during lactation than cows fed the low RUP prepartum diets followed by the basal lactation diet plus AA. In contrast, cows receiving the

high RUP prepartum diets followed by the basal lactation diet lost more body condition than cows receiving the high RUP prepartum diet and fed the AA supplemented lactation diet.

Dry Matter Intake

There were no effects of prepartum diet on DMI for the last 10 d of gestation (Figure 1). Intakes of DM decreased in a linear fashion from 10 d to 3 d prepartum and averaged 14.5 kg/d.

Dry matter intakes averaged 21.1 kg for the first 70 DIM (Table 4). There was a prepartum RUP by lactation diet interaction for DMI during lactation (Table 4); AA supplementation during lactation increased DMI when cows had received the high RUP prepartum diets but not the low RUP prepartum diets.

Milk and Milk Composition Responses

There were no effects of prepartum diets and no prepartum diet by lactation diet interaction on milk yield (Table 4). However, there was a tendency for inclusion of Lys and Met in the prepartum diets to decrease milk yield when the basal lactation diet was fed but not when the lactation diet included supplemental AA. Inclusion of AA in the lactation diet decreased milk yields (44.5 vs. 46.6 kg/d).

Milk fat percentages were not influenced by dietary treatments (Table 4). This observation is consistent with other studies that have fed AA or infused Lys and Met into the duodenum of cows in early lactation (1, 14, 15, 28, 29). Percentages of CP and true protein in milk were greatest when the high RUP prepartum diets were fed (3.06 vs 2.96 % for CP and true protein, respectively) compared to the low RUP prepartum diets (2.91 and 2.79 %

for CP and true protein, respectively). There were no effects of including AA in prepartum diets and no prepartum RUP by prepartum AA interactions on percentages of CP and true protein in milk. Feeding Lys and Met during lactation increased mean concentrations of milk CP (3.10 vs. 2.93 %) and true protein (2.93 vs 2.77 %). However, increases in concentrations of milk CP and true protein as a result of postpartum AA supplementation were greatest when high RUP prepartum diets were fed (+.26 and +.23 percentage units) vs. when the low RUP prepartum diets were fed (+.09 and +.10 percentage units) and when AA were not included in the prepartum diets (+.25 and +.23 percentage units) vs. when they were included in the prepartum diets (+.10 and +.10 percentage units). Interestingly, cows fed the low RUP prepartum diet plus AA did not respond with greater milk protein percentages during lactation, whereas cows fed the other prepartum diets did respond. The prepartum RUP by lactation diet interactions for concentrations of milk CP and true protein are shown in Figure 3 and 4, respectively. Cows receiving the high RUP prepartum diets and the basal lactation diet plus AA had greater percentages of milk CP and true protein throughout the 10 wk of lactation (Figure 3 and 4). There were no significant effects of dietary treatments on concentration of milk urea N (Table 4) or yields of milk fat, CP, or true protein (Table 5).

Discussion

Chemical Composition of the Diets

Prepartum and lactation diets are shown in Tables 1 and 2. Soybean products (solvent-extracted soybean meal, SoyPass™, and roasted soybeans) were used as protein supplements to provide desired contents of RDP and RUP in diet DM; SoyPass™ was used

as the primary source of supplemental RUP. Protein supplements were limited to soybean products to ensure a consistent pattern of AA in supplemental protein across treatments and because the RUP fraction of soybean products tend to have greater intestinal digestibilities than other high RUP supplements such as dried distiller's grains and animal by-products (37). For example, Stern et al (37) estimated the intestinal digestibilities of RUP in solvent extracted soybean meal, SoyPass™, hydrolyzed feather meal, batch-dried blood meal, and meat and bone meal to be 90 %, 88 %, 67 %, 63 % and 55 %, respectively.

It is understood that the quantity of microbial CP passing to the small intestine will be compromised if RUP supplementation replaces required RDP. A shortage of RDP in the diet decreases ruminal availability of N for microbial protein synthesis (6, 37). Since the AA profile of microbial protein closely matches the AA profile of milk and tissue protein and because microbial protein has a high intestinal digestibility (≥ 90 %), maximizing ruminal synthesis and passage to the small intestine of microbial protein is important to maximizing milk and milk protein production (6). In order to evaluate the response of prepartum cows to RUP in this experiment (4.9 and 6.6 % of DM for the low RUP and high RUP diets, respectively), RDP concentrations were kept constant at 9.0 % of diet DM for both prepartum diets.

Body Weight and Body Condition Score

Cows fed the high RUP prepartum diets lost less weight and body condition during the first 10 wk of lactation than did cows fed the low RUP prepartum diets. Van Saun et al (39) reported similar findings with first lactation Holstein cows. They attributed the response to greater accumulation of body tissue reserves while cows were in late gestation. Ruegg et

al (25) suggested that cows calving with more body condition lose more weight in the first 8 wk of lactation than thinner cows and that cows with more body condition will respond to postpartum dietary RUP with greater milk yields; this appears to be the case with the cows fed the low RUP prepartum diets in this experiment. Seymour and Polan (30) also proposed that efficiency of use of adipose tissue reserves for milk production is improved by increasing dietary RUP. In our experiment, the greater loss of body condition during lactation by the cows fed the low RUP prepartum diets followed by the supplemented lactation diet appears to be related to greater body condition. This suggests there is an interaction between an improvement in intestinal AA balance and the efficiency of use of adipose tissue reserves.

Dry Matter Intake

The apparent effects of greater DMI during the transition period in response to increased prepartum dietary concentrations of RUP appears to be related to an increase in a cow's ability to adapt to high protein diets after calving (38). Schwab et al (28) reported increased intake with infusion of Lys and Met into the duodenum of early lactation cows. They concluded that one benefit of meeting AA requirements by improving intestinal balance of AA was an increase in feed intake. Additionally, Van Saun et al (38) suggested that improving maternal protein reserves during late gestation by feeding diets with greater RUP concentration may be a stimulant for DMI, and for greater milk and milk protein synthesis in the subsequent lactation.

Increasing maternal protein reserves by feeding higher RUP diets and by feeding rumen-stable forms of Lys and Met during the transition period appears to provide additional endogenous AA for both gluconeogenesis and for milk protein synthesis (22, 24, 38).

Meeting more adequately the requirements for AA with increased dietary CP, RUP, and supplemental AA during the transition period also may improve lipid metabolism. The effect of increased absorbable AA on lipid metabolism in early lactation appears to be a reduction in liver lipid accumulation as well as an improvement in efficiency of use of adipose tissue reserves for milk synthesis (22, 25, 30). Since the accumulation of lipid in the liver has been related to decreased DMI, decreased lactational performance, and increased incidence of ketosis in early lactation, it appears that decreased liver triacylglycerol accumulation during the periparturient period may stimulate appetite (4, 38, 41).

Milk and Milk Composition Responses

Milk yield responses ranging from increases to decreases have been reported as a result of increasing RUP concentrations in prepartum diets (7, 38, 42). Reasons for these variable responses may be similar to the reasons for the variable responses reported for increased dietary RUP during early lactation. Part of the variation can be attributed to the amount and AA composition of feed protein reaching the small intestine. This variation in the balance of absorbable AA from dietary sources could also reduce or eliminate a milk yield response to supplemental Lys and Met (1). Hoffman et al (17) reported that early lactation cows did not respond to additional RUP when provided by expeller soybean meal due to higher than predicted RUP reaching the small intestine as the result of greater than expected RUP content of solvent soybean meal. Guillaume et al (11) also reported no milk yield response to increasing RUP with extruded soybeans and supplemental Lys and Met. They concluded that there was no response to rumen-stable AA because of higher than estimated dietary RUP passage to the small intestine and lower than estimated RDP in diets containing

extruded soybeans (11). Therefore, it is possible that the diets in our experiment contained an excess of digestible RUP, and that imbalances of Lys and Met in total essential AA were greater than predicted. It appears that the early lactation diets provided excess absorbable AA relative to other nutrients (i.e., energy), and that energy limited milk yield responses to supplemental Lys and Met (12, 15, 17, 18). This observation is consistent with the observation of others who have infused individual AA or casein into the duodenum (12, 13, 14, 15). Guinard and Rulquin (12) reported a linear increase in milk yield and milk protein content when incremental amounts of casein were infused into the duodenum of lactating cows, but only an increase in milk protein content when incremental amounts of Lys or Met were infused (12, 13). They reasoned that the carbon chains of casein were used for ATP synthesis and thereby provided additional metabolizable energy for milk synthesis, whereas metabolizable energy limited milk yield response when only Lys or Met were infused (12).

Greater milk CP content in cows previously fed supplemental Lys and Met, suggests there is an interaction between prepartum protein nutrition and milk CP response to Lys and Met in early lactation. However, the nature of this interaction is unknown. Perhaps the response to AA supplementation during early lactation is related to a greater supply of endogenous AA to support milk production, thereby sparing dietary AA for milk protein synthesis.

The increase in milk protein content can be attributed to true protein, because milk urea N was not different between the prepartum and postpartum dietary treatments. Concentrations of milk urea N (Table 4) did not decrease with the addition of Lys and Met to the lactation diet. This observation was unexpected because Linn and Olson (21) indicated

that milk urea N values decrease when intestinal AA balance is improved. The interaction between prepartum RUP and early lactation diet is shown in Figure 4. Variations in milk urea N reflect the variation in DMI in the first 10 wk of lactation, and variable intake may be the reason for no significant treatment effects. Gustafsson and Palmquist (16) indicated that milk urea N values may not accurately evaluate efficiency of N utilization in high yielding early lactation cows. They reasoned that the effects of diet concentrations of N on N utilization in early lactation may be masked by variable intakes and large milk volumes associated with early lactation (16).

Several studies have reported increases in milk crude and true protein contents with diet supplementation or duodenal infusion of Lys and Met (1, 14, 28, 29). The results of these experiments indicate that Lys and Met are the two most limiting AA for milk protein synthesis when corn-based diets are fed, and that milk protein response is a good indicator of AA adequacy. Therefore, increased milk CP and true protein percentages when rumen-stable forms of Lys and Met were fed indicate that the basal diet did not supply adequate Lys and Met for milk protein synthesis. Schwab et al (28, 29) and Guinard and Rulquin (14) concluded that the extent of Lys and Met limitation is greatest during early lactation (4 to 8 wk) when milk yield is greatest and that milk protein responses to improvements in Lys and Met nutrition are greatest during early lactation. From studies in which incremental amounts of Lys or Met were duodenally infused during early lactation, Schwab et al (28, 29) and Guinard and Rulquin (15) concluded that Lys and Met concentrations in duodenal digesta must be equal to or greater than 15.2 % and 5.2 % of total essential AA, respectively to attain the maximum milk protein concentrations. Furthermore, the results of our experiment

indicate that early lactation milk protein responses to supplemental Lys and Met appears to be influenced by prepartum dietary RUP and Lys and Met supplementation.

Conclusions

Results from this experiment indicate that milk protein content in early lactation is affected by prepartum RUP concentration. Increasing concentration of RUP in prepartum diets and increasing concentration of Lys and Met in RUP of postpartum diets are effective ways of increasing milk protein content of early lactation cows.

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TABLE 1. Ingredient composition of basal diets.

Ingredient	Prepartum diet		Lactation diet
	Low RUP	High RUP	
	(% of DM)		
Corn silage[1]	40.1	38.5	30.3
Haycrop silage[2]	16.5	14.0	14.2
Ground shelled corn	29.6	28.5	26.2
Soybean hulls	4.2	5.0	6.1
Soybean meal, solvent, 48% CP	3.4	1.3	9.0
Roasted soybeans	---	---	4.6
Treated soybean meal[3]	3.3	10.2	4.6
Fat[4]	0.9	0.7	1.3
Minerals and vitamins[5]	2.0	1.9	3.9

[1] Treated at ensiling with .4 to .5 % urea. Contained 32.9 % DM, and as a percent of DM 42.7 NDF, 26.8 ADF, and 10.9 CP.

[2] Contained 35.2 % DM, and as a percent of DM 56.7 NDF, 39.4 ADF, and 17.2 CP.

[3] SoyPass™ (Lignotech USA, Inc., Overland Park, KS).

[4] Alifet™ (Alifet USA, Inc., Cincinnati, OH).

[5] Contained: 4.8 % Ca, 0.0 % P, 14.5 % Mg, .01 % K, 4.8 % Na, 7.5 % Cl, 5.8 % S, 79.3 ppm Co, 427 ppm Cu, 438 ppm Fe, 899 ppm Mn, 20 ppm Se, 1388 ppm Zn, 133 KIU/kg vitamin A, 33 KIU/kg vitamin D, and 831 KIU/kg vitamin E for the prepartum diets. Contained: 17.7 % Ca, 2.3 % P, 4.1 % Mg, .06 % K, 8.1 % Na, 3.25 % Cl, 2.1 % S, 40 ppm Co, 224 ppm Cu, 2718 ppm Fe, 479 ppm Mn, 7 ppm Se, 725 ppm Zn, 27 KIU/kg vitamin A, 7 KIU/kg vitamin D, and 168 KIU/kg vitamin E for the lactation diet.

TABLE 2. Chemical composition of consumed diets [1] and estimates of duodenal digesta lysine and methionine.

Chemical composition	Prepartum diet		Lactation diet
	Low RUP	High RUP	
	(% of DM)		
CP	13.8	15.6	18.1
RUP [2]	4.9	6.6	7.3
RDP [2]	8.9	9.0	10.8
RUP (% of CP)	35.5	42.0	40.2
NSC [3]	42.4	42.4	38.0
NE_L [2], Mcal/kg DM	1.8	1.8	1.8
NDF	33.1	30.8	28.4
ADF	19.8	19.2	16.1
EE	4.2	4.2	5.2
Ca	.37	.35	1.0
P	.22	.28	.51
Mg	.35	.35	.30
K	1.5	1.5	1.5
S	.27	.28	.21
Na	.15	.15	.36
Cl	.39	.39	.33
Vitamin A (x 1000 IU)	.63	.58	2.9
Vitamin D (x 1000 IU)	.16	.15	0.7
Vitamin E (x 100 IU)	3.9	3.6	17.9
Amino acids in duodenal digesta [4, 5], % of EAA			
Lys	14.9	15.1	14.9
Met	4.3	4.2	4.3

[1] Chemical composition of the consumed diets were calculated by dividing the difference between the quantities of offered and refused nutrients by DMI.

[2] Calculated from NRC (22).

[3] Non-structural carbohydrates = 100% - (% NDF + % CP + % EE + % ash).

[4] Calculated using the regression equations of Socha (1994) where Lys= 14.43 - (0.04 x RUP, % of CP) - (0.29 x CP, % of DM) + (0.54 x RUP-Lys, % of RUP-EAA) + (-.13) and where Met = 5.36 - (0.08 x RUP, % of CP) + (3.94 x RUP-Met, % of CP) + (-.15).

[5] Values for Lys and Met do not include Trp as part of EAA.

TABLE 3. Least square means for body weight (BW), body weight change, body condition score (BCS), and body condition change the first 70 days in milk as influenced by prepartum and lactation diets.

	Prepartum diet				Effect					
	Low RUP	Low RUP + AA	High RUP	High RUP + AA	RUP[1]	AA[2]	RUP x AA	LD[3]	LD x RUP	LD x AA
BW, kg										
Basal lactation diet[4]	641 (13)[6]	642 (12)	631 (15)	641 (14)	ns[7]	ns	ns	ns	ns	ns
Basal lactation diet + AA[5]	615 (15)	629 (13)	651 (13)	643 (15)						
BCS										
Basal lactation diet	3.0 (.2)	3.1 (.2)	2.7 (.2)	2.8 (.2)	ns	ns	ns	ns	.06	ns
Basal lactation diet +AA	2.7 (.2)	2.8 (.2)	3.0 (.2)	2.9 (.2)						
Body weight change										
Basal lactation diet	-131 (14)	-114 (13)	-118 (16)	86 (15)	.06	ns	ns	ns	ns	ns
Basal lactation diet +AA	-139 (16)	-107 (14)	- 96 (14)	109 (15)						
Body condition change										
Basal lactation diet	-.71 (.13)	-.68 (.12)	-.73 (.14)	-.69 (.14)	ns	ns	ns	ns	.07	ns
Basal lactation diet +AA	-.87 (.16)	-.98 (.13)	-.61 (.12)	-.58 (.14)						

[1] Prepartum dietary crude protein .

[2] Prepartum amino acids.

[3] Lactation diet.

[4] For cows receiving the basal diet, n= 9, 9 ,7, and 10 for the low RUP, low RUP+AA, high RUP, and high RUP+AA prepartum diets, respectively.

[5] For cows receiving the basal plus AA diet, n= 9, 9 ,7, and 10 for the low RUP, low RUP+AA, high RUP, and high RUP+AA prepartum diets, respectively.

[6] Standard errors are in parentheses.

[7] Means were considered non-significant (ns) at $P<0.10$.

TABLE 4. Least square means for DMI, milk yield, and milk component percentages during the first 70 days in milk as influenced by prepartum and lactation diets. [1]

	Prepartum diet				Effects					
	Low RUP	Low RUP + AA	High RUP	High RUP +AA	RUP[2]	AA[3]	RUP x AA	LD[4]	LD x RUP	LD x AA
DMI, kg/d										
Basal lactation diet[5]	21.4 (.6)[7]	21.0 (.6)	20.4 (.7)	19.9 (.6)	ns[8]	ns	ns	.06	.02	ns
Basal lactation diet +AA[6]	20.7 (.7)	21.2 (.6)	21.7 (.6)	22.3 (.7)						
Milk, kg/d										
Basal lactation diet	48.5 (1.4)	46.5 (1.3)	47.4 (1.6)	43.8 (1.5)	ns	ns	ns	.08	ns	ns
Basal lactation diet +AA[6]	44.5 (1.7)	44.4 (1.4)	44.3 (1.4)	44.7 (1.6)						
Milk fat, %										
Basal lactation diet	3.77 (.10)	3.69 (.10)	3.88 (.12)	3.73 (.12)	ns	ns	ns	ns	ns	ns
Basal lactation diet +AA	3.92 (.12)	3.86 (.10)	3.82 (.11)	3.83 (.12)						
Milk CP, %										
Basal lactation diet	2.84 (.06)	2.99 (.05)	2.91 (.07)	2.96 (.06)	.02	ns	ns	.000	.06	.09
Basal lactation diet +AA	3.04 (.07)	2.97 (.06)	3.20 (.06)	3.18 (.06)						
Milk true protein, %										
Basal lactation diet	2.67 (.06)	2.81 (.05)	2.78 (.07)	2.81 (.06)	.02	ns	ns	.000	ns	ns
Basal lactation diet +AA	2.88 (.07)	2.80 (.06)	3.03 (.06)	3.01 (.06)						
Milk urea N, mg/dl										
Basal lactation diet	15.2 (.6)	13.8 (.6)	14.6 (.7)	15.0 (.7)	ns	ns	ns	ns	ns	ns
Basal lactation diet + AA	14.1 (.7)	14.8 (.6)	14.1 (.6)	14.5 (.7)						

[1]First lactation milk yield and initial BW at 21 d before expected calving were covariates for DMI and milk yield. First lactation milk fat and CP percentages and initial BW at 21 d prior before calving were covariates for percentages of milk fat and CP, and first lactation milk crude protein percentage and BW at 21 d before expected calving were covariates for milk true protein percentages and milk urea N.

[2] Prepartum dietary RUP.

[3] Prepartum amino acids.

[4] Lactation diet.

[5] For cows receiving the basal lactation diet, n= 9, 9, 7, and 10 for the low RUP, low RUP+AA, high RUP, and high RUP+AA prepartum diets, respectively.

[6] For cows receiving the basal lactation diet plus AA, n= 9, 9, 7, and 10 for the low RUP, low RUP+AA, high RUP, and high RUP+AA prepartum diets, respectively.

[7] Standard errors are in parentheses.

[8] Means were considered non-significant at $P>0.10$.

TABLE 5. Least square means for milk fat, crude protein and true protein yields the first 70 days in milk as influenced by prepartum and lactation diets. [1]

	Prepartum diet				Effects					
	Low RUP	Low RUP +AA	High RUP	High RUP +AA	RUP[2]	AA[3]	RUP x AA	LD[4]	LD x RUP	LD x AA
Milk fat yield, g/d										
Basal lactation diet[5]	1796 (71)[7]	1665 (67)	1781 (81)	1595 (79)	ns[8]	ns	ns	ns	ns	ns
Basal lactation diet + AA[6]	1794 (81)	1695 (70)	1639 (73)	1685 (79)						
Milk CP yield, g/d										
Basal lactation diet	1351 (40)	1355 (37)	1332 (46)	1281(43)	ns	ns	ns	ns	ns	ns
Basal lactation diet + AA	1415 (47)	1314 (40)	1364 (40)	1359(43)						
Milk true protein yield, g/d										
Basal lactation diet	1273 (36)	1278 (34)	1265 (42)	1215 (39)	ns	ns	ns	ns	ns	ns
Basal lactation diet + AA	1340 (43)	1221 (37)	1306 (36)	1293 (39)						

[1]First lactation milk yield and initial BW at 21 d before expected calving were covariates for DMI and milk yield. First lactation milk fat and CP percentages and initial BW at 21 d prior before calving were covariates for percentages of milk fat and CP, and first lactation milk crude protein percentage and BW at 21 d before expected calving were covariates for milk true protein percentages and milk urea N.

[2] Prepartum dietary RUP.

[3] Prepartum amino acids.

[4] Lactation diet.

[5] For cows receiving the basal lactation diet, n= 9, 9, 7, and 10 for the low RUP, low RUP+AA, high RUP, and high RUP+AA prepartum diets, respectively.

[6] For cows receiving the basal lactation diet plus AA, n= 9, 9, 7, and 10 for the low RUP, low RUP+AA, high RUP, and high RUP+AA prepartum diets, respectively.

[7] Standard errors are in parentheses.
[8] Means were considered non-significant at $P<0.10$.

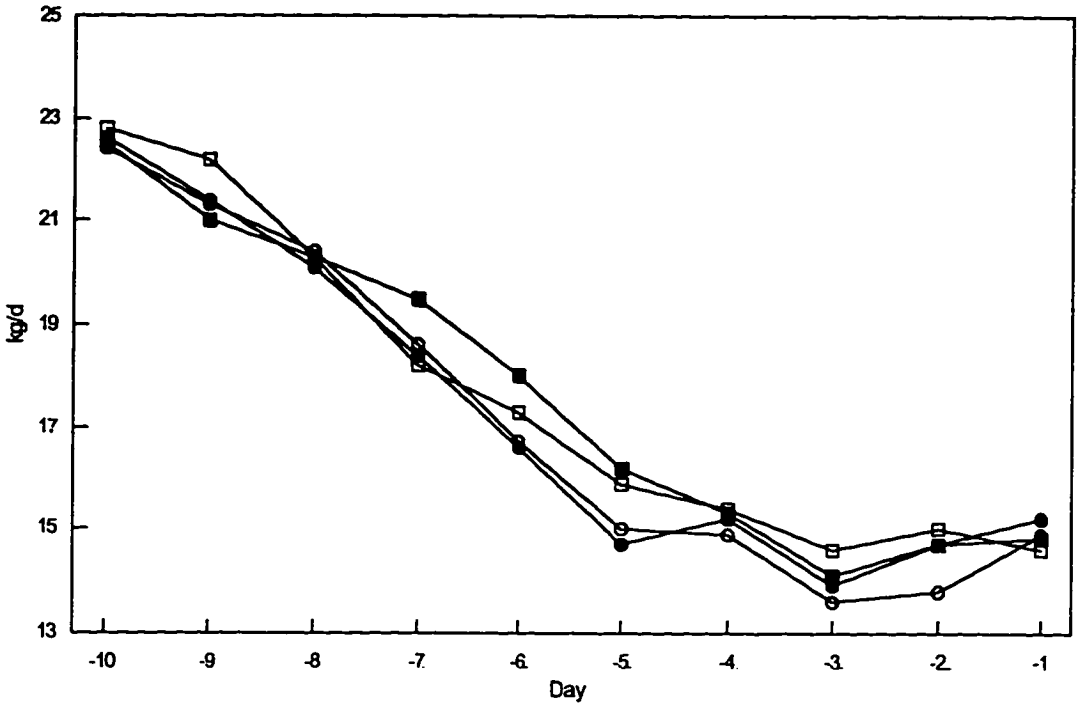

Figure 1. Least square means for dry matter intake for the last 10 d of gestation. The means are for the prepartum treatments; high RUP (■), high RUP diet plus AA (□), low RUP diet (●), and low RUP prepartum diet plus AA (○).

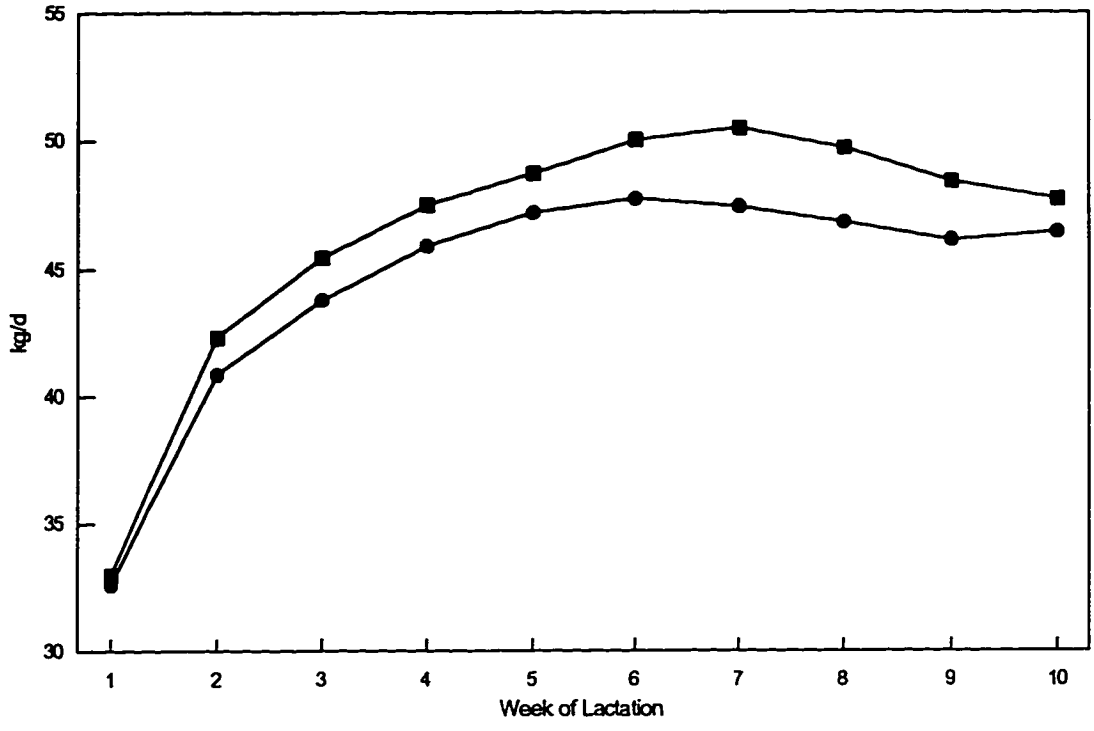

Figure 2. Least square means for milk yield during the first 70 days in milk for cows fed the basal lactation diet (■) and the basal lactation diet plus Lys and Met (●).

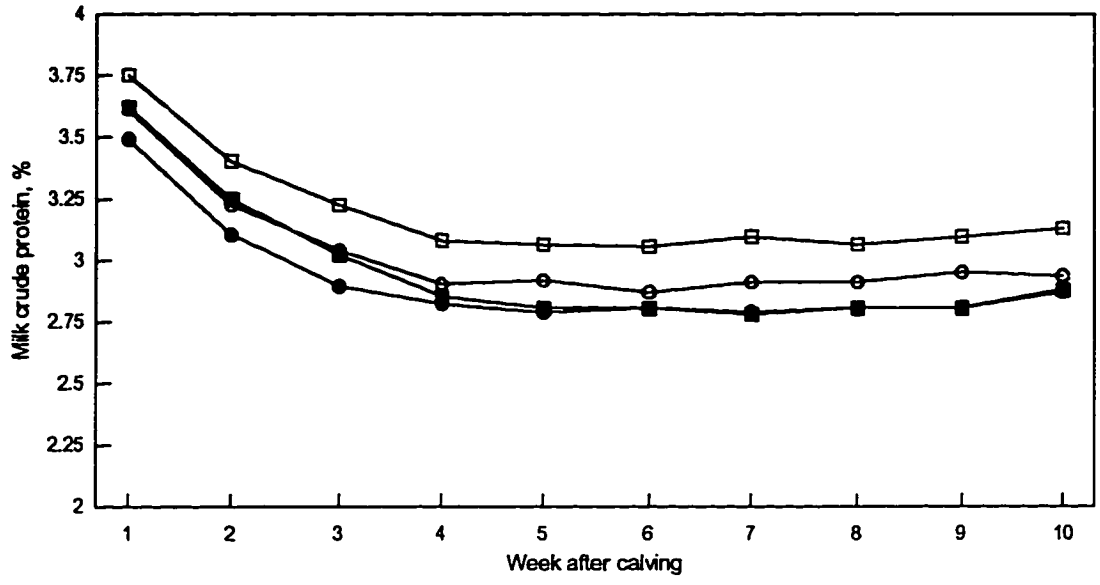

Figure 3. Least square means for milk CP percentages for the first 70 DIM. The means are the interactions of prepartum RUP and lactation diet treatments; high RUP prepartum diet and basal lactation diet (■), high RUP prepartum diet and basal lactation diet plus AA (□), low RUP prepartum diet and basal lactation diet (●), and low RUP prepartum diet and basal lactation diet plus AA (○).

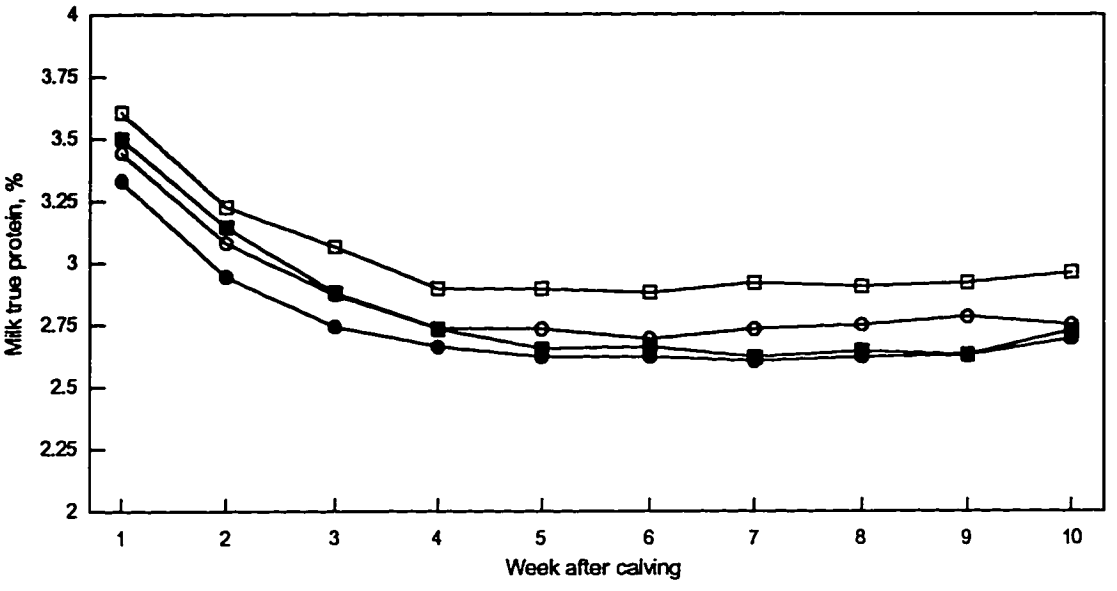

Figure 4. Least square means for milk true percentages for the first 70 DIM. The means are the interactions of prepartum RUP and lactation diet treatments; high RUP prepartum diet and basal lactation diet (■), high RUP prepartum diet and basal lactation diet plus AA (□), low RUP prepartum diet and basal lactation diet (●), and low RUP prepartum diet and basal lactation diet plus AA (○).

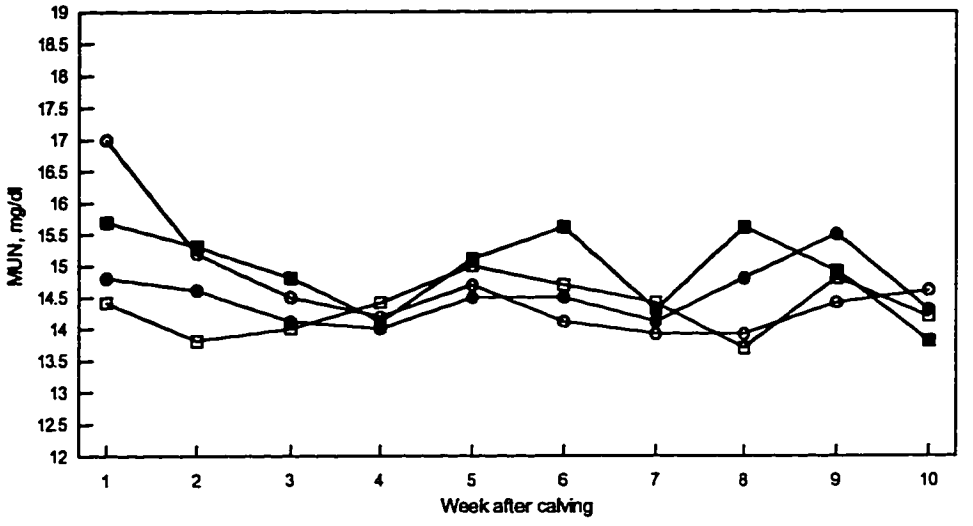

Figure 5. Least square means for milk urea N (MUN) for the first 70 DIM. The means are the interactions of prepartum RUP and lactation diet treatments; high RUP prepartum diet and basal lactation diet (■), high RUP prepartum diet and basal lactation diet plus AA (□), low RUP prepartum diet and basal lactation diet (●), and low RUP prepartum diet and basal lactation diet plus AA (○).

CHAPTER IV

OPTIMIZING LYSINE AND METHIONINE NUTRITION DURING THE PERIPARTURIENT AND POSTPARTURIENT PERIODS. II. BLOOD METABOLITES AND HORMONES

Abstract

Seventy-two multiparous cows were blocked by expected calving date and assigned to a 2 x 2 factorial arrangement of four diet treatments beginning 21 d before expected calving. The prepartum diets were (% of DM) low RUP(4.9 % RUP and 13.8 % CP), low RUP plus 5.2 g/d of rumen-stable Lys and 4.5 g/d of rumen-stable Met, high RUP (6.6 % RUP and 15.6 % CP), and high RUP plus 4.6 g/d of rumen-stable Lys and 8.3 g/d of rumen-stable Met. Each block contained eight cows and two cows per block received each of the prepartum dietary treatments. On the day of calving, one cow from each pair received a basal lactation diet (7.3 % RUP and 18.1 % CP) and the other cow received the basal diet plus 12.9 g/d of rumen-stable Lys and 19.5 g/d of rumen-stable Met. Diets were corn-based and all supplemental protein was provided by soybean products. Cows remained on treatments until 70 d postpartum. Beginning 10 d before expected calving through 7 d of lactation, blood was sampled daily from the tail vein. From wk 2 until wk 10 of lactation, blood was sampled three times per week and combined for weekly analysis of glucose and nonesterified fatty acids. Urine was sampled following the same scheme as blood, and ketone concentrations were measured. Plasma samples from the first fifty-six cows on the experiment were analyzed for

growth hormone, insulin, and prolactin. Prepartum dietary treatment and lactation diet did not influence plasma glucose, nonesterified fatty acid, and urine ketone concentrations. Postpartum amino acid supplementation of cows fed the high RUP prepartum diets increased postpartum glucose concentrations, reduced concentration of nonesterified fatty acids in plasma, and reduced urine ketone concentrations compared to cows fed the low RUP prepartum diets. Growth hormone and prolactin concentrations were not significantly affected by pre- or postpartum dietary treatments, prepartum insulin concentration was significantly greater during the last 10 d of gestation when cows were fed the high RUP prepartum diet. Feeding greater concentrations of dietary RUP prepartum decreased postpartum nonesterified fatty acids and urine ketone concentrations and increased plasma glucose concentration. There appears to be an affect of prepartum RUP content on subsequent lactational responses of blood metabolites and hormones.

(**Key words:** Lysine, methionine, periparturient, and postparturient)

Abbreviation key: **AA**= amino acids, **BCS**= body condition score, **BW**= body weight, **CP**= crude protein, **DMI** = dry matter intake, **GH** = growth hormone, **Lys** = lysine, **Met** = methionine, **RDP**= ruminally degradable protein, **RUP**= ruminally undegradable protein

Introduction

The metabolic adaptations associated with the periparturient period include increased adipose tissue lipolysis, increased hepatic gluconeogenesis, and increased amino acid (**AA**) N release from skeletal muscle (7, 9, 22). These adaptations occur in response to changes in nutrient demand, diminished feed intake, and in response to alterations in the hormonal signals

of growth hormone (**GH**), insulin, and prolactin (7, 9). Growth hormone appears to be the primary stimulus for altering liver, adipose tissue, and skeletal muscle metabolism. Additionally, responses to insulin and prolactin appear to be influenced by GH mediated changes in these tissues (7, 9, 16, 22, 26, 27).

As parturition approaches, plasma concentrations of GH increase with peak concentrations occurring at parturition (9). Elevated GH concentrations near parturition appears to be responsible for increased lipolytic rates and insulin resistance in adipose tissue which begin about 1 wk before calving and continue through 21 d of lactation (25). The increase in lipolysis is indicated by increased plasma concentrations of nonesterified fatty acids (**NEFA**) (7, 10, 25). Plasma concentrations of NEFA are directly related to NEFA uptake by the liver, and hepatic rates of fatty acid esterification and oxidation increase in response to elevated plasma concentrations of NEFA and GH (6, 25). Increased lipid accumulation in the liver and increased rates of ketogenesis have been noted in transition cows as early as 1 wk prepartum (6, 10).

The liver responds to alterations in GH concentrations during the periparturient period with increasing rates of gluconeogenesis and NEFA oxidation (15, 21, 27). Grum et al (16) evaluated the effect of energy density of prepartum diets on hepatic lipid metabolism and plasma hormone concentrations; diets contained either 1.27 Mcal or 1.44 Mcal/ kg NE_L. Plasma concentrations of GH were greater when the high energy diet was fed whereas plasma insulin concentrations and liver triacylglycerol concentrations were reduced (15). The authors attributed the decrease in liver lipids to an increase in hepatic fatty acid oxidation, a response

to elevated GH and reduced insulin concentrations (16).

Simmons et al (27) provided evidence that the administration of GH during the periparturient period also increases N retention; indicated by elevated ratios of concentrations of serum urea N to urine N-methyl histidine. They concluded that GH increases N retention during the periparturient period by increasing cellular uptake of AA and decreasing cellular protein degradation (27). The effect of increased N retention during late gestation on subsequent increases in milk yield appears to result because of increased gluconeogenic substrate (i.e., amino acids) to support the lactational demands for glucose (9).

Although the exact role that prolactin plays in metabolic adaptations during the periparturient period is not well understood, prolactin does appear to enhance both mammogenesis and lactogenesis. Prolactin in combination with GH is essential for mammary growth and function; increasing prepartum concentrations of plasma prolactin and GH by arginine infusion increased subsequent milk yield by 10 % (13). Chew (13) proposed that arginine infusion increased prolactin and GH secretion by stimulating pituitary and hypothalamic activity. They attributed the increase in milk yield to prolactin and GH acting synergistically to increase mammary gland secretory cell growth and number (13).

The primary objective of this experiment was to determine the effects of, and interaction between, feeding a greater amount of RUP and supplemental Lys and Met during the last 3 wk of gestation on plasma concentrations of glucose, NEFA, insulin, GH, and prolactin. A secondary objective was to determine the effects of supplemental Lys and Met during the first 10 wk of lactation on the same metabolites. All of the supplemental protein

in the corn-based pre- and postpartum diets was provided by soybean products.

Materials and Methods

Experimental Design and Treatments

All procedures related to animal care were conducted with the approval of the University of New Hampshire Institutional Animal Care and Use Committee. Seventy-two multiparous cows were blocked by expected calving date and assigned to a 2 x 2 factorial arrangement of dietary treatments beginning 21 d before expected calving. The four treatments were: 1) low RUP (4.9 %of DM; 13.8 % CP) no Lys and Met; 2) low RUP plus 5.2 g/d of Lys and 4.5 g/d of Met; 3) high RUP (6.6 % of DM; 15.6 % of CP) no Lys and Met; and 4) high RUP plus 4.6 g/d of Lys and 8.3 g/d of Met. Lysine and Met were provided in rumen-stable forms (Smartamine™ M and Smartamine™ ML, Rhône-Pôulenc Animal Nutrition, Atlanta, GA) and were fed in amounts to achieve intestinal concentrations of 15.5 and 5.5% of total essential AA for Lys and Met, respectively, as estimated by the regression equations of Socha (35). Each block contained eight cows and two cows per block received each of the prepartum diets. On the day of calving, one cow from each pair within a block received the basal lactation diet and the other cow received the basal lactation diet plus 12.9 g/d of rumen-stable Lys and 19.5 g/d of rumen-stable Met (Table 1). Cows remained on the lactation diet until 70 DIM. Three cows were removed from the experiment; two due to environmental mastitis and one because of an abomasal ulcer.

Feeding and Management of Cows

Diets (Tables 1 and 2) were fed as a TMR and were prepared by weighing each ingredient and mixing in a drum type mixer (Data Ranger; American Calan, Inc., Northwood, NH). Amounts of lignosulfonate and heat-treated soybean meal (SoyPass®, Ligno Tech USA, Inc., Overland Park, KS) and solvent-extracted soybean meal were adjusted in the diets to achieve RUP and RDP concentrations in diet DM of 4.9 and 8.9 % and 6.6 and 9.0 %, respectively, for the low and high RUP prepartum diets; the basal lactation diet was formulated to contain 7.3 % RUP and 10.8 % RDP. Cows were fed 50 % of the total daily allotment at 1400 h, 30 % at 2000 h, and 20 % at 0400 h. Rumen-stable AA were topdressed at the time of feeding, with 50 % at 1400 h, 30 % at 2000 h, and 20 % at 0400 h.

Measurements, Collection, and Analysis of Samples

Procedures as described previously (33) were followed for feed and ort sampling and for feed analysis. After a 5-d adjustment period to the prepartum diets, blood was sampled by venipuncture of the coccygeal vein approximately 3 h after the morning feeding on d 16 prepartum; the concentrations of plasma metabolites in this sample were used as a covariate in the analysis of pre-and postpartum blood samples.

Beginning 10 d before expected calving and continuing through 7 d postpartum, blood was sampled daily from the coccygeal vein 3 h post-feeding. From wk 2 through wk 10 postpartum, blood was sampled three times per week and equal aliquots of plasma were composited weekly before analysis. Blood was collected into two 10-ml evacuated tubes

containing EDTA (Vacutainer®, Becton Dickinson, Rutherford, NJ). Tubes were placed immediately on ice until they were centrifuged at 3,000 *x g* for 10 min at 5°C. One aliquot of plasma was harvested and stored (-20°C) for glucose determination (Glucose Trinder, Sigma Diagnostics, St. Louis, MO) and another for analysis of NEFA (Wako Chemical, Neuss Germany); samples were stored at -20°C until analysis. Urine was sampled following the same time-table as the blood samples; acetoacetic acid concentrations were determined using Ketostix® (Miles Inc., Elkhart, IN.). Plasma from the first 56 cows assigned to the experiment was analyzed for GH, prolactin, and insulin using radioimmunoassay procedures (2). The GH data for 2 blocks (16 cows) were discarded because of assay error.

Statistical Analysis and Calculations

Repeated measurements for plasma metabolites were reduced to total experimental prepartum and postpartum means. Postpartum glucose, NEFA, GH, insulin, prolactin, and urine ketones values represent the least square means of the three-way interaction of prepartum RUP, prepartum AA, and lactation diet. The GLM procedure of SAS (25) was used to analyze the data, and means were considered different at $P<.10$.

The model for analysis was a randomized block design with a 2 x 2 factorial arrangement of treatments. Total first lactation milk yield and initial BW (21 d before calving) were covariates for glucose, NEFA, GH, insulin, prolactin, and ketone. Initial blood and urine samples (17 d prepartum) were also used as covariates for blood metabolites, hormones, and urine ketones.

Results

Plasma Glucose

Plasma glucose concentrations are shown in Table 3 and Figure 1. Glucose concentrations remained rather constant from 10 d to 2 d before calving (Figure 1). Glucose concentrations increased sharply the day before calving and peaked at calving. After calving, glucose decreased to below prepartum concentrations by d 7 of lactation.

There was significant prepartum RUP by prepartum AA by lactation diet interaction on postpartum concentrations of glucose (Table 3 and Figure 1). Supplementing the lactation diet with Lys and Met decreased plasma glucose in cows previously fed either of the low RUP prepartum diets, increased plasma glucose in cows previously fed the unsupplemented, high RUP prepartum diet, and had no effect on plasma glucose in cows previously fed the AA supplemented , high RUP prepartum diet.

Plasma NEFA

Plasma NEFA concentrations are presented in Table 3 and Figure 2. Plasma NEFA began to increase 2 d before parturition and peaked on the day of calving (Figure 2). After calving, NEFA concentrations increased with the rate of decrease being faster in the first 3 wk of lactation and slower from wk 3 through wk 10 of lactation at which time concentrations had returned to prepartum concentrations. There were no effects of prepartum diet treatments on prepartum NEFA concentrations, however, there was a significant prepartum dietary RUP by lactation diet interaction effect on postpartum NEFA concentrations (Table 3 and Figure 2). Particularly during the first 2 wk of lactation and to

a lesser extent thereafter, supplementing the lactation diet with Lys and Met decreased plasma NEFA concentrations in cows previously fed the high RUP prepartum diets but tended to either increase (first 7 d of lactation) or have no effect on plasma NEFA in cows previously fed the low RUP diets (Figure 3).

Urine Ketones

Urine ketone concentrations remained relatively constant during the prepartum period and ranged from 10 to 23 mg/dl (Figure 3). The range of urine ketone during the prepartum period are similar to those reported by others (4) and are considered to be with in the normal range for ruminants. After calving, ketone concentrations in urine increased to moderate levels (20 to 45 mg/dl), peaked at 6 d of lactation and from d 7 until d 70 of lactation ketone concentrations in urine remained above prepartum concentrations. There were no effects of prepartum diet treatments on prepartum ketone concentrations, however, there was a significant prepartum dietary RUP by lactation diet interaction effect on postpartum ketone concentrations (Table 3 and Figure 2). Particularly during the first 2 wk of lactation and to a lesser extent thereafter, supplementing the lactation diet with Lys and Met decreased urine ketone concentrations in cows previously fed the high RUP prepartum diets but tended to either increase (first 7 d of lactation) or have no effect on urine ketone in cows previously fed the low RUP diets (Figure 3).

Plasma Insulin

Prepartum concentration of insulin in plasma ranged from 1250 to 2000 pg/ml. Two d before calving, insulin concentrations began to decrease and concentrations were lowest

on the day of calving. After calving, insulin concentration increased slightly, but was below prepartum concentration. There was a significant effect of prepartum dietary RUP on prepartum insulin concentration (Table 4 and Figure 4). Cows fed the high RUP diets had greater insulin concentration prepartum compared to cows fed the low RUP diets. Mean insulin concentration for the last 10 d of gestation were 1563 pg/ml and 1284 pg/ml for the high RUP and low RUP diets, respectively.

Plasma Growth Hormone

Growth hormone concentration in plasma ranged from 5 to 8 ng/ml from d 10 until d 2 before calving, when concentration began to increase and peak GH occurred between 4 and 7 d postpartum (Figure 5). Concentrations of plasma GH remained elevated through the first 5 wk of lactation and were about twice prepartum concentrations. Similar trends and concentrations of GH have been reported in multiparous cows from 2 wk prepartum until 10 wk postpartum (17). Growth hormone concentrations were not influenced by pre- or postpartum dietary treatments (Table 4).

Plasma Prolactin

Plasma prolactin concentration began to increase 3 d prior to parturition and peaked 1 d before calving for cows fed the high RUP prepartum diets and on the day of calving for cows that received the low RUP prepartum diets (Figure 6). Mean concentrations 1 d before calving were significantly affected by prepartum RUP and were 62 ng/ml for cows receiving the low RUP diets and 42 ng/ml for cows fed the high RUP diets. By d 4 of lactation, prolactin concentration in plasma returned to prepartum concentrations. Prepartum RUP

tended to increase prolactin concentration prepartum, but did not effect postpartum prolactin concentration (Table 4) during the first 10 wk of lactation.

Discussion

The changes in plasma glucose concentrations that occurred over time during the periparturient period in this experiment agree with the results of previous studies (30, 31, 32). Vazquez-Anon et al (31) and Studer et al (30) reported sharp increases in glucose concentrations as cows approached parturition and attributed the changes to increased hepatic gluconeogenesis and glycogenolysis in response to diminished feed intake and increased catecholamine and glucocorticoid concentrations.

Mean prepartum concentrations of plasma glucose were greater in this experiment (75 to 85 mg/dl) (Figure 1) than those reported previously (65 to 70 mg/dl) (30, 31, 32). Prepartum diets contained high non-structural carbohydrate (NSC) (42.4 % of diet DM) and the high NSC concentration may have been responsible for the elevated glucose concentrations. In a review of recommendations for feeding periparturient dairy cows, Grummer (18) suggested that increasing prepartum intake of fermentable carbohydrate may increase plasma glucose concentrations. He reasoned that an increase in dietary fermentable carbohydrates will increase ruminal propionate production and propionate in turn will increase hepatic glucose synthesis (18).

The inconsistent responses of glucose to AA supplementation during early lactation are difficult to explain. Others (19, 20) have not reported a glucose response to improved

Lys and Met nutrition. Guinard and Rulquin (19, 20), infused incremental amounts of Lys (0, 9, 27, and 63 g/d) or Met (0, 8, 16, and 32 g/d) into the duodenum of mature, midlactation Holstein cows. They reported no change in arterial glucose concentrations and no increase in efficiency of glucose extraction from blood by the mammary gland. Results from this experiment suggest Lys and Met supplementation of early lactation cows previously fed the high RUP prepartum diet increases glucose concentration, perhaps by stimulation of glucose synthesis. The increase in glucose synthesis may be related to an increase in gluconeogenic AA. McNeill et al (22) suggested that increasing prepartum protein concentration may improve the supply of endogenous AA available to support early lactation. Greater endogenous AA would than spare dietary AA for other functions.

Concentrations of NEFA in plasma are an indicator of adipose tissue mobilization (10, 18, 30, 31). Fatty acids are mobilized from adipose tissue in late gestation and early lactation in response to decreased feed intake and changes in hormonal signals associated with parturition and lactation. Plasma NEFA concentrations from d 10 to d 2 prepartum in our experiment (100 to 200 mEq/l) are considerably lower than the concentrations (350 to 500 mEq/l) reported by others (27, 31, 32) over the same prepartum period of time. The differences between our results and the results of others is undoubtedly attributable to the energy density of our prepartum diets (1.8 Mcal NE_L per kg of DM). Cows fed high energy (1.44 Mcal NE_L per kg of DM) prepartum diets during the last 10 wk of gestation had NEFA concentrations between 100 to 150 mEq/l during the last 2 wk gestation (16).

The increase in NEFA concentrations beginning 2 d before calving indicates that the

cows experienced negative energy balance prior to the onset of lactation. Others have reported similar responses (10, 16, 30, 31, 32). Vazquez et al (31) attributed the increases in NEFA concentrations to diminished intake and an increase in lipolytic hormones. Additionally, Simmons et al (27) and Grum et al (16) paralleled the increase in NEFA concentrations in the final week of gestation to an increase in GH and a decrease in insulin concentration. Similar changes in plasma concentrations of GH and insulin in this experiment relative to NEFA concentrations were observed in our experiment.

The response in plasma NEFA concentrations to Lys and Met supplementation in early lactation appears to related to an improved intake during the first 10 wk of lactation. For example, the cows fed the AA supplemented lactation diet had greater intake (22.0 kg/d; Figure 7) than cows receiving the unsupplemented diet (20.0 kg/d), when both groups were previously fed the high RUP prepartum diet. Socha et al (28) reported increased DMI and decreased plasma NEFA concentrations in early lactation cows (14 to 40 d in milk) that were infused duodenally with a fixed amount of Lys (10 g/d) and incremental amounts of Met (0, 3.5, 7, 10.5, and 16.0 g/d). Intakes were highest and plasma NEFA concentrations were lowest at the higher levels of infused Met. The authors attributed the decrease in NEFA to a reduction in fat mobilization. In our experiment, it seems likely that fatty acid mobilization was greater in cows receiving the basal lactation diet, since milk yields were highest (Figure 8) but DMI was lowest (Figure 7).

During the periparturient period, when the rate of hepatic fatty acid oxidation is high, the liver synthesizes considerable quantities of the ketones acetoacetate, acetone, and 3-

hydroxybutyrate (4). This is reflected in elevated blood and urine concentrations of acetoacetate and 3-hydroxybutyrate. Ketones are either utilized by extrahepatic tissues (i.e., mammary gland), respired (acetone), or eliminated via urine (3-hydroxybutyrate and acetoacetate) (23). Normal concentrations of blood and urine ketones in lactating cow are between 10 and 15 mg/dl, moderate concentrations are 40 to 60 mg/dl, and clinical ketosis occurs when concentrations exceed 80 mg/dl (1).

Postpartum feeding of Lys and Met when cows were previously fed the high RUP prepartum diets decreased urine ketone concentrations but increased urine ketones in cows that had consumed the low RUP prepartum diets. These differences appear to be due to the differences in plasma NEFA concentrations. Aeillo et al (1) reported that hepatic ketogenesis increased as plasma NEFA concentrations and hepatic uptake of NEFA increases. Therefore, it stands to reason that dietary treatments during early lactation that decrease NEFA concentration would also result in decreased ketone synthesis and vice versa.

Prepartum insulin concentrations were greater in cows fed the high RUP diets than in cows fed the low RUP diets. The diet differences resulted in greater intakes of metabolizable protein for cows fed the high RUP diets, since prepartum DMI was similar for both groups (14.6 and 14.5 kg/d for the low RUP and high RUP diets, respectively). Chew et al (12) reported a trend for greater insulin concentrations 2 wk before calving when cows consumed 0.95 kg/d CP vs. 0.75 kg/d during the last 4 wk of gestation. The authors offered no explanation for the influence of dietary CP on plasma insulin. Bines and Hart (11) indicated that greater intakes of concentrate feeds stimulate insulin secretion in early lactation

cows, however, they did not distinguish between carbohydrate and protein intake.

The primary regulator of nutrient partitioning during late gestation and early lactation appears to be GH (7, 9). Prepartum and lactation dietary treatments did not influence GH concentration in this experiment. The lack of a treatment effect on GH may have been due to lower numbers of samples analyzed for GH than for the other hormones.

The exact mechanism by which prolactin stimulates milk synthesis is unknown, however the proposed mechanism of action appears to be the stimulation of mammary cell growth and differentiation during the periparturient period (2). Akers et al (2) reported decreased mammary alveolar cell numbers and increased numbers of undifferentiated cells in multiparous cows 10 d before parturition following injection of CB154 beginning 14 d before parturition. They attributed the decrease in cell numbers and the increase in undifferentiated cells to the inhibition of prolactin secretion.

Previously reported (2) changes in prolactin concentrations indicated that as parturition approaches prolactin concentrations remain constant and average about 30 ng/ml, but by 2 d prepartum, concentrations increase rapidly with peak concentrations occurring at parturition (194 ng/ml). Prolactin concentrations returned to prepartum values (28 ng/ml) by 3 d in milk. The changes in plasma concentrations of prolactin reported by Akers et al (2) agree with these in this experiment; however peak concentrations at calving were only 70 ng/ml in our experiment (Figure 6). Dietary treatments did not affect prolactin concentrations in this experiment.

The changes in prolactin concentrations associated with the periparturient period may

also influence nutrient partitioning. Bell (9) indicated that prolactin may inhibit the actions of insulin on adipose tissue, resulting in greater mobilization of fatty acids. Prolactin may also influence the partitioning of absorbed AA between the liver and extrahepatic tissues. More likely, prolactin and GH act synergistically to increase lactogenesis and partitioning of nutrients to the mammary gland (13).

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TABLE 1. Ingredient composition of basal diets.

Ingredient	Prepartum diet		Lactation diet
	Low RUP	High RUP	
	(% of DM)		
Corn silage[1]	40.1	38.5	30.3
Haycrop silage[2]	16.5	14.0	14.2
Ground shelled corn	29.6	28.5	26.2
Soybean hulls	4.2	5.0	6.1
Soybean meal, solvent, 48% CP	3.4	1.3	9.0
Roasted soybeans	---	---	4.6
Treated soybean meal[3]	3.3	10.2	4.6
Fat[4]	0.9	0.7	1.3
Minerals and vitamins[5]	2.0	1.9	3.9

[1] Treated at ensiling with .4 to .5 % urea. Contained 32.9 % DM, and as a percent of DM 42.7 NDF, 26.8 ADF, and 10.9 CP.

[2] Contained 35.2 % DM, and as a percent of DM 56.7 NDF, 39.4 ADF, and 17.2 CP.

[3] SoyPass™ (Lignotech USA, Inc., Overland Park, KS).

[4] Alifet™ (Alifet USA, Inc., Cincinnati, OH).

[5] Contained: 4.8 % Ca, 0.0 % P, 14.5 % Mg, .01 % K, 4.8 % Na, 7.5 % Cl, 5.8 % S, 79.3 ppm Co, 427 ppm Cu, 438 ppm Fe, 899 ppm Mn, 20 ppm Se, 1388 ppm Zn, 133 KIU/kg vitamin A, 33 KIU/kg vitamin D, and 831 KIU/kg vitamin E for the prepartum diets. Contained: 17.7 % Ca, 2.3 % P, 4.1 % Mg, .06 % K, 8.1 % Na, 3.25 % Cl, 2.1 % S, 40 ppm Co, 224 ppm Cu, 2718 ppm Fe, 479 ppm Mn, 7 ppm Se, 725 ppm Zn, 27 KIU/kg vitamin A, 7 KIU/kg vitamin D, and 168 KIU/kg vitamin E for the lactation diet.

TABLE 2. Chemical composition of consumed diets [1] and estimates of duodenal digesta lysine and methionine.

	Prepartum diet		Lactation
Ingredient	Low RUP	High RUP	diet
	(% of DM)		
CP	13.8	15.6	18.1
RUP [2]	4.9	6.6	7.3
RDP [2]	8.9	9.0	10.8
RUP (% of CP)	35.5	42.0	40.2
NSC [3]	42.4	42.4	38.0
NE_L [2], Mcal/kg DM	1.8	1.8	1.8
NDF	33.1	30.8	28.4
ADF	19.8	19.2	16.1
EE	4.2	4.2	5.2
Ca	.37	.35	1.0
P	.22	.28	.51
Mg	.35	.35	.30
K	1.5	1.5	1.5
S	.27	.28	.21
Na	.15	.15	.36
Cl	.39	.39	.33
Vitamin A (x 1000 IU)	.63	.58	2.9
Vitamin D (x 1000 IU)	.16	.15	0.7
Vitamin E (x 100 IU)	3.9	3.6	17.9
Amino acids in duodenal digesta [4, 5], % of EAA			
Lys	14.9	15.1	14.9
Met	4.3	4.2	4.3

[1] Chemical composition of the consumed diets was calculated by dividing the difference between the quantities of offered and refused nutrients by DMI.

[2] Calculated from NRC (22).

[3] Non-structural carbohydrates = 100% - (% NDF + % CP + % EE + % ash).

[4] Calculated using the regression equations of Socha (1994) where Lys= 14.43 - (0.04 x RUP, % of CP) - (0.29 x CP, % of DM) + (0.54 x RUP-Lys, % of RUP-EAA) + (-.13) and where Met = 5.36 - (0.08 x RUP, % of CP) + (3.94 x RUP-Met, % of CP) + (-.15).

[5] Values for Lys and Met do not include Trp as part of EAA.

TABLE 3. Least square means for concentrations of plasma glucose and NEFA and for urine ketone during the first 70 d of lactation.[1]

	Prepartum diet				Effects						
	Low RUP	Low RUP +AA	High RUP	High RUP +AA	RUP[2]	AA[3]	RUP x AA	LD[4]	LD x RUP	LD x AA	LD x RUP x AA
Glucose, mg/dl											
Basal lactation diet[5]	75 (2)[6]	78 (2)	67 (3)	72 (3)	ns[7]	ns	ns	ns	.004	ns	.03
Basal lactation diet +AA[5]	69 (3)	74 (2)	78 (2)	71 (3)							
NEFA, mEq/l											
Basal lactation diet	223 (27)	200 (26)	286 (36)	260 (33)	ns	ns	ns	ns	.09	ns	ns
Basal lactation diet + AA	255 (33)	176 (29)	201 (28)	201 (32)							
Urine ketone, mg/dl											
Basal lactation diet	22 (3)	23 (3)	30 (4)	24 (4)	ns	ns	ns	ns	.04	ns	ns
Basal lactation diet+AA	27 (4)	29 (3)	25 (3)	20 (3)							

[1] Plasma concentration of glucose, nonesterified fatty acids, growth hormone, insulin, and prolactin in a sample taken 16 d before expected calving were covariates for concentrations of the same metabolites in samples taken thereafter. First lactation milk yield, initial BW at 21 d before expected calving, and samples taken at 16 d before expected calving were covariates for glucose, NEFA, and urine ketone.

[2] Prepartum dietary RUP.

[3] Prepartum amino acids.

[4] Lactation diet.

[5] For cows receiving the each of the two lactation diets, n= 7, 7, 5, and 8 for the low RUP, low RUP+AA, high RUP, and high

RUP+AA prepartum diets, respectively.

[6] Standard errors are in parentheses.

[7] Means were considered non-significant at $P>0.10$.

TABLE 4. Least square means for plasma concentrations of growth hormone, prolactin, and insulin during the first 70 d of lactation.[1]

	Prepartum diet				Effects					
Item	Low RUP	Low RUP +AA	High RUP	High RUP +AA	RUP[2]	AA[3]	RUP x AA	LD[4]	LD x RUP	LD x AA
Growth hormone, ng/ml										
Basal lactation diet[5]	9 (1)[7]	10 (1)	12 (2)	12 (1)	ns[7]	ns	ns	ns	ns	ns
Basal lactation diet +AA[5]	11 (2)	12 (1)	12 (1)	10 (2)						
Insulin, pg/ml										
Basal lactation diet	891 (63)	889 (55)	1012 (81)	1008 (68)	.08	ns	ns	ns	ns	ns
Basal lactation diet + AA	851 (79)	924 (60)	955 (62)	928 (67)						
Prolactin, ng/ml										
Basal lactation diet	27 (31)	10 (30)	16 (45)	21 (33)	ns	ns	ns	ns	ns	ns
Basal lactation diet +AA	137 (38)	24 (32)	17 (30)	7 (33)						

[1] Plasma concentration of glucose, nonesterified fatty acids, growth hormone, insulin, and prolactin in a sample taken 16 d before expected calving were covariates for concentrations of the same metabolites in samples taken thereafter. First lactation milk yield, initial BW at 21 d before expected calving, and samples taken at 16 d before expected calving were covariates for glucose, NEFA, and urine ketone.

[2] Prepartum dietary RUP.

[3] Prepartum amino acids.

[4] Lactation diet.

[5] For cows receiving each of the lactation diets, n= 7, 7, 5, and 8 for the low RUP, low RUP+AA, high RUP, and high RUP+AA prepartum diets, respectively.

[6] Standard errors are in parentheses.

[7] Means were considered non-significant at $P>0.10$.

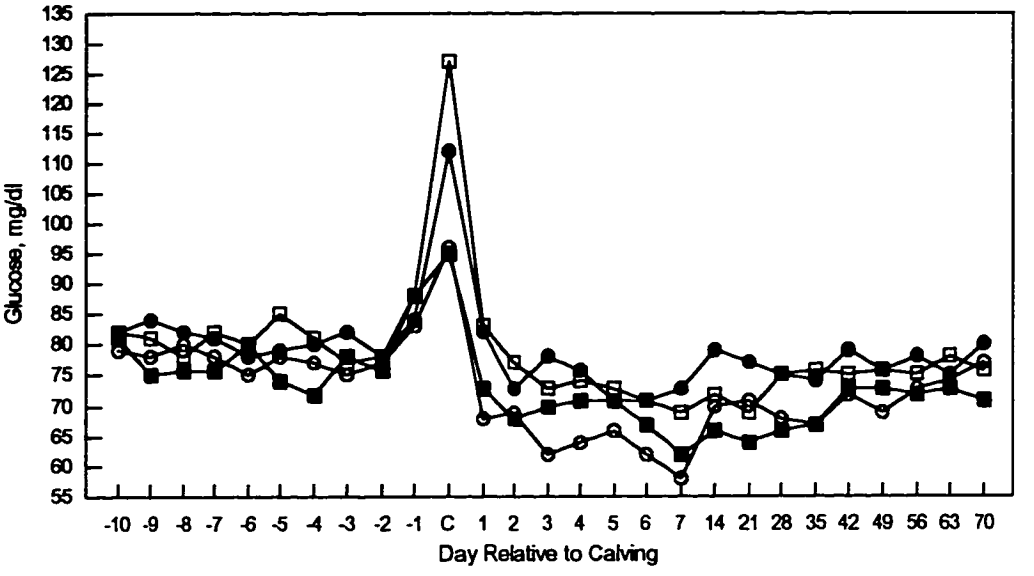

Figure 1. Least square means for plasma glucose from 10 d before calving through 70 DIM. The means are the interactions of prepartum RUP and lactation diet treatments; high RUP prepartum diet and basal lactation diet (■), high RUP prepartum diet and basal lactation diet plus AA (□), low RUP prepartum diet and basal lactation diet (●), and low RUP prepartum diet and basal lactation plus AA (○).

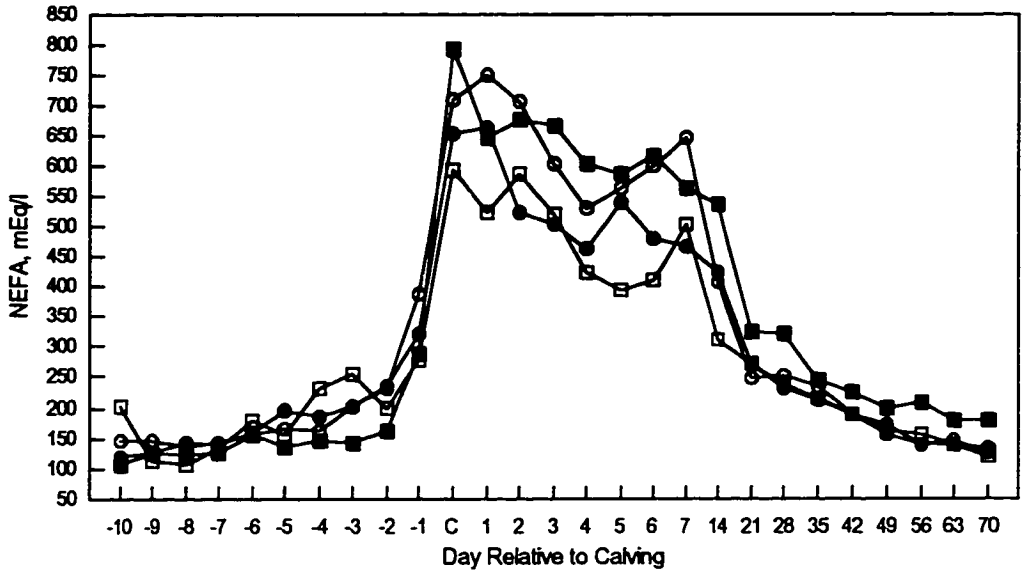

Figure 2. Least square means for plasma NEFA concentrations from 10 d before calving through 70 DIM. The means are the interactions of prepartum RUP and lactation diet treatments; high RUP prepartum diet and basal lactation diet (■), high RUP prepartum diet and basal lactation diet plus AA (□), low RUP prepartum diet and basal lactation diet (●), and low RUP prepartum diet and basal lactation plus AA (○).

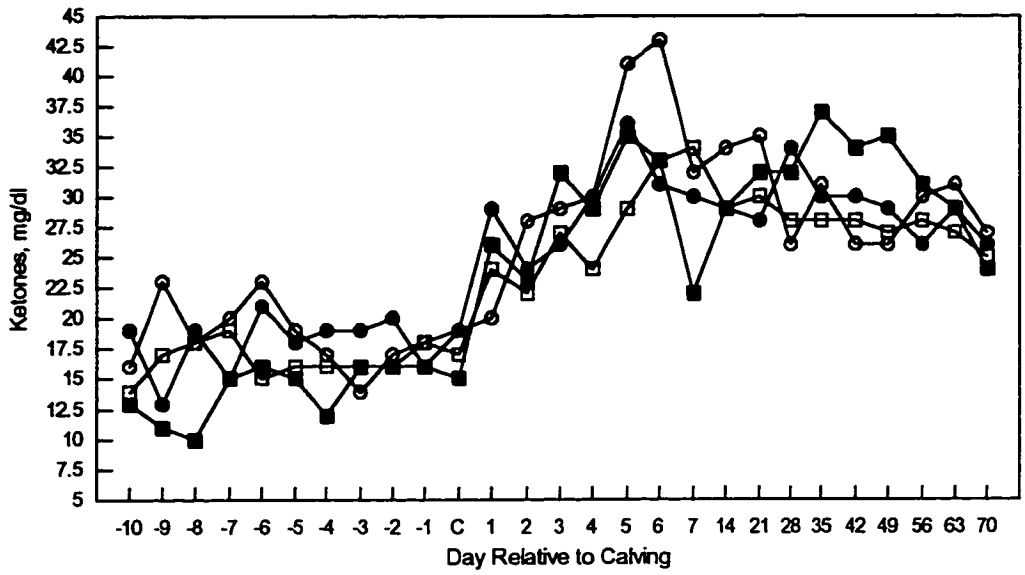

Figure 3. Least square means for urine ketones from 10 d before calving through 70 DIM. The means are the interactions of prepartum RUP and lactation diet treatments; high RUP prepartum diet and basal lactation diet (■), high RUP prepartum diet and basal lactation diet plus AA (□), low RUP prepartum diet and basal lactation diet (●), and low RUP prepartum diet and basal lactation plus AA (○).

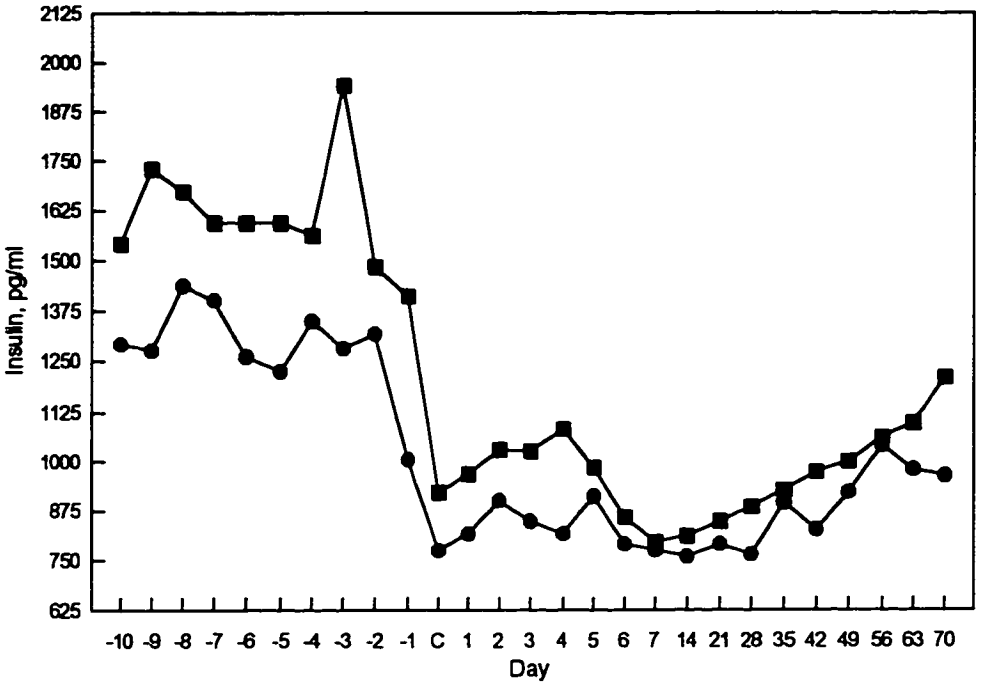

Figure 4. Least square means for plasma insulin concentrations. The means are for prepartum RUP treatments; high RUP prepartum diet (■) and low RUP prepartum diet (●).

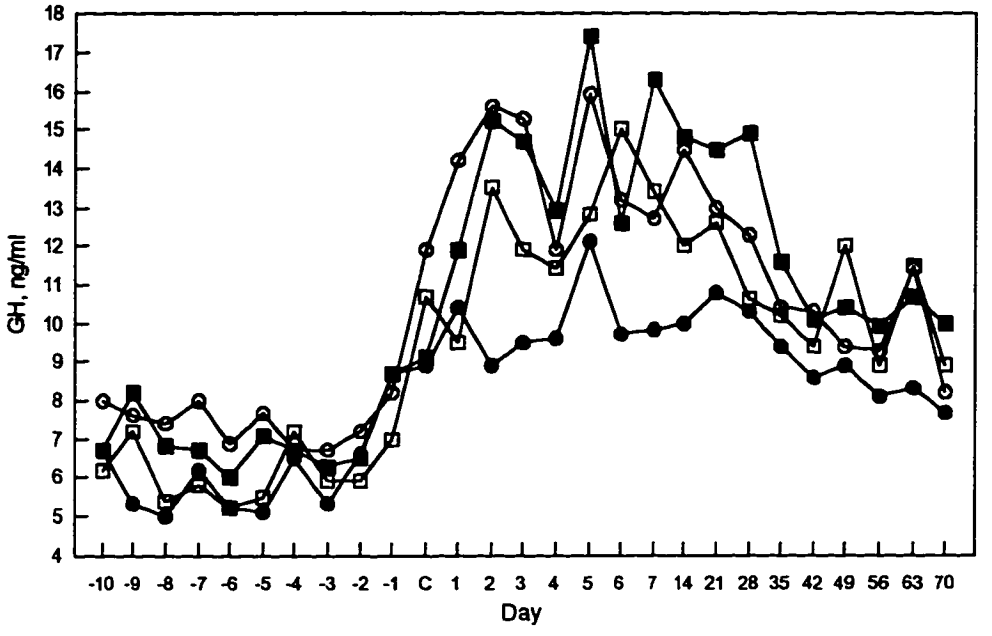

Figure 5. Least square means for plasma growth hormone concentrations from 10 d before calving through 70 DIM. The means are the interactions of prepartum RUP and lactation diet treatments; high RUP prepartum diet and basal lactation diet (■), high RUP prepartum diet and basal lactation diet plus AA (□), low RUP prepartum diet and basal lactation diet (●), and low RUP prepartum diet and basal lactation plus AA (○).

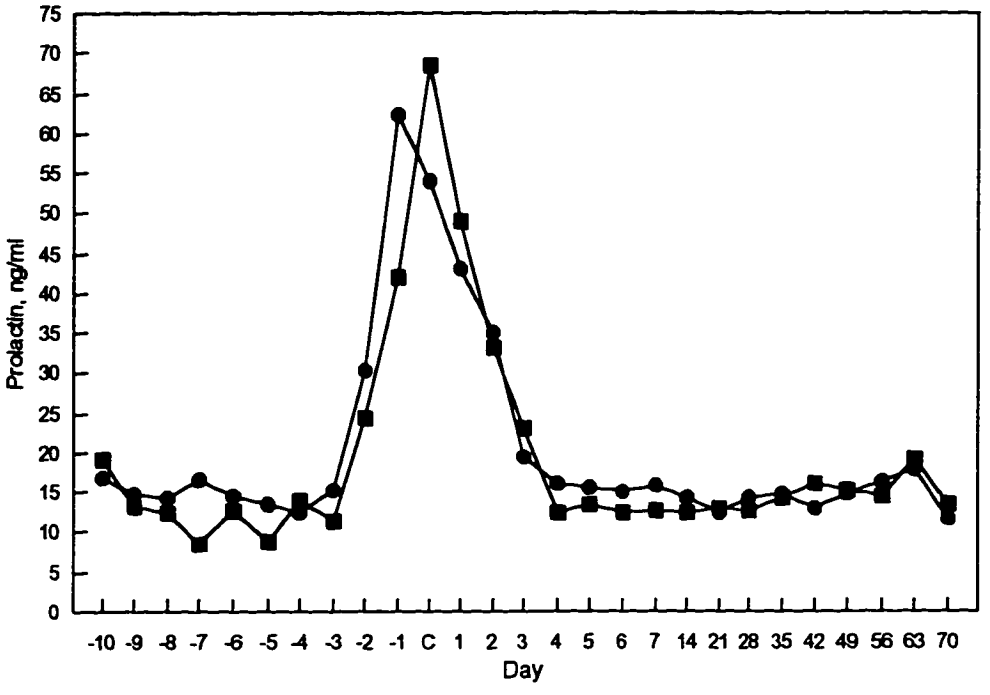

Figure 6. Least square means for plasma prolactin concentrations. The means are for prepartum RUP treatments ; high RUP prepartum diet (●) and low RUP prepartum diet (■).

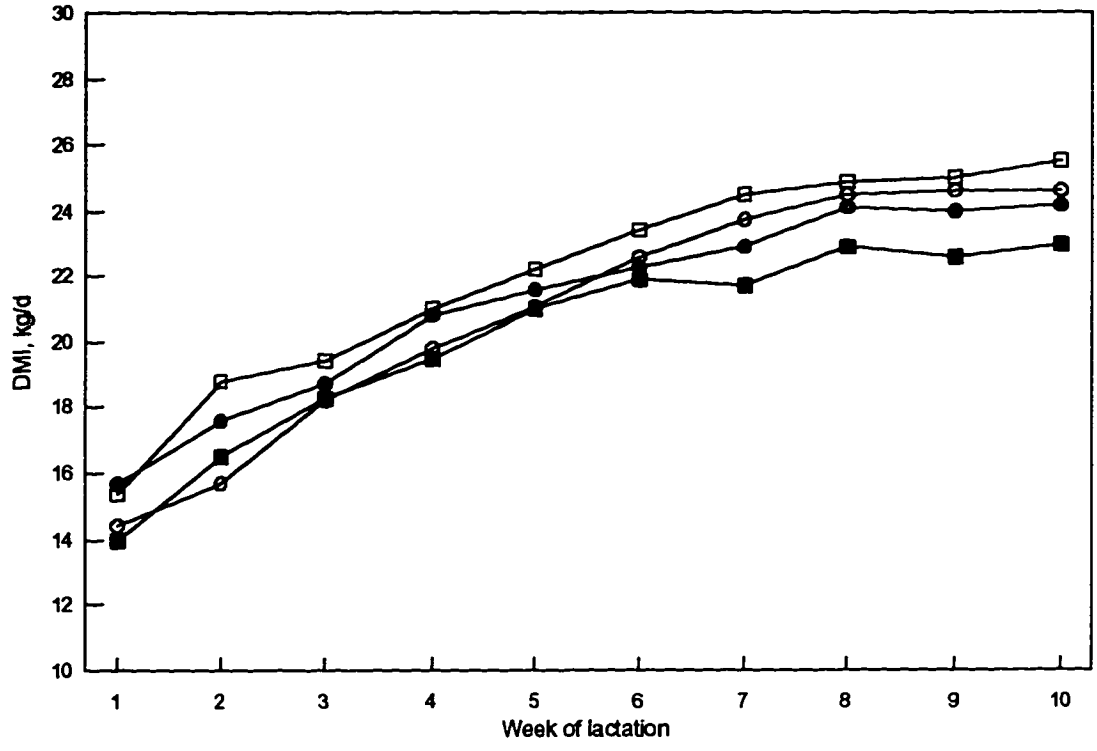

Figure 7. Least square means for DMI during the first 70 DIM. The means are the interactions of prepartum RUP and lactation diet treatments; high RUP prepartum diet and basal lactation diet (■), high RUP prepartum diet and basal lactation diet plus AA (□), low RUP prepartum diet and basal lactation diet (●), and low RUP prepartum diet and basal lactation plus AA (○).

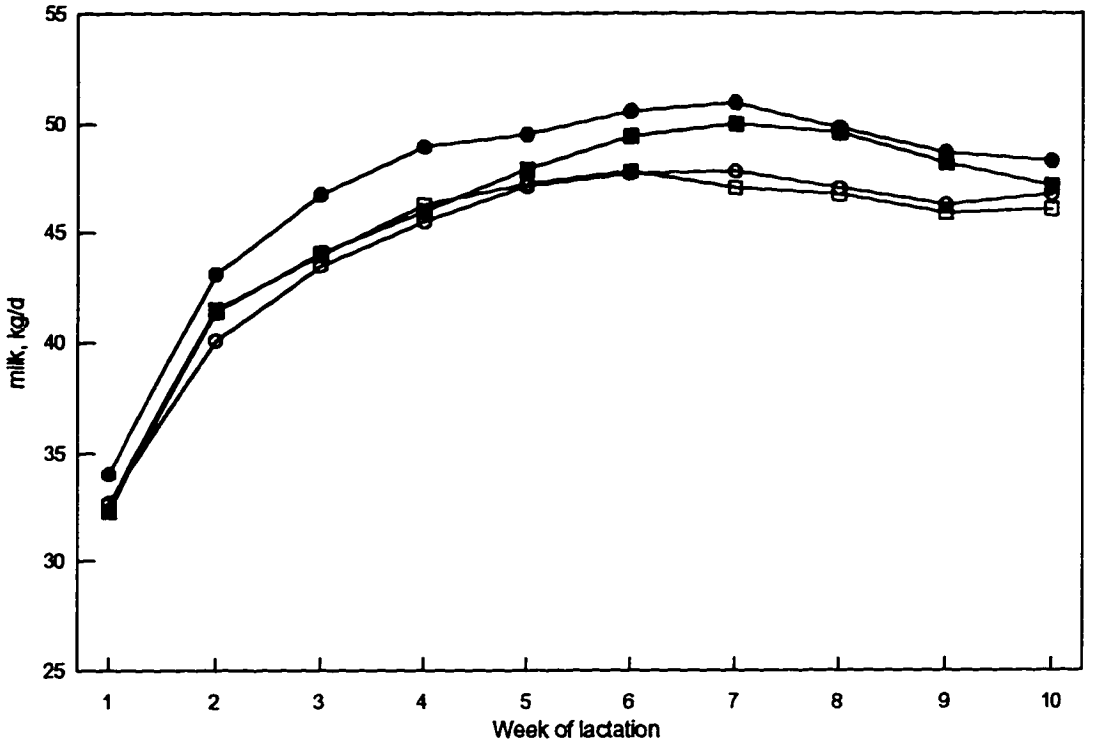

Figure 8. Least square means for milk yield during the first 70 DIM. The means are the interactions of prepartum RUP and lactation diet treatments; high RUP prepartum diet and basal lactation diet (■), high RUP prepartum diet and basal lactation diet plus AA (□), low RUP prepartum diet and basal lactation diet (●), and low RUP prepartum diet and basal lactation plus AA (○).

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APPENDIX

TABLE 1. Analysis of variance table for dry matter intake (DMI), body weight (BW), body condition score (BCS), plasma glucose, nonesterified fatty acids (NEFA) growth hormone (GH), insulin, and prolactin during the last 10 d of gestation.

		Mean Squares							
Source	DF	DMI	BW	BCS	Glucose	NEFA	GH	Insulin	Prolactin
Block	8	9	4912	0	164	7446	1	586240	175
Cov [1]	1	1	8654	1	1	763	0	145476	45
RUP [2]	1	5	6341	1	9	4404	0	1671262	362
AA [3]	1	1	1027	0	2	6873	0	476770	34
RUP x AA	1	5	2077	0	67	3124	0	1091993	3

[1] First lactation milk yield data was a covariate for dry matter intake, body weight, and body condition score. Plasma concentration of glucose, nonesterified fatty acids, growth hormone, insulin, and prolactin in a sample taken 17 d before expected calving were covariates for concentrations of the same metabolites in samples taken thereafter.

[2] Prepartum dietary RUP.

[3] Prepartum amino acids.

TABLE 2. Analysis of variance table for dry matter intake (DMI), body weight (BW), body condition score (BCS), milk yield, milk fat (MF), milk crude protein (MCP), milk true protein (MTP), and milk urea nitrogen (MUN) during the first 70 d of lactation

		Mean Squares							
Source	DF	DMI	BW	BCS	Milk	MF	MCP	MTP	MUN
Block	8	11.2	2478	0.1	52.4	0.4	0.1	0.1	12.4
Cov [1]	1	20.3	344	0.5	279.2	1.9	0.2	0.2	5.6
BW [1]	1	1.8	22074	1.0	232.5	0.8	0.1	0.1	0.1
RUP [2]	1	0.0	1364	0.1	6.7	0.0	0.2	0.2	0.1
AA [3]	1	0.1	275	0.0	41.0	0.1	0.0	0.0	0.0
RUP x AA	1	0.0	173	0.1	0.0	0.0	0.0	0.0	2.6
LD [4]	1	11.0	318	0.1	56.1	0.1	0.5	0.4	1.4
RUP x LD	1	16.9	3677	1.0	8.5	0.1	0.1	0.1	0.8
AA x LD	1	3.9	27	0.1	44.6	0.0	0.1	0.1	4.5
RUP x AA x LD	1	0.1	1103	0.0	1.2	0.0	0.0	0.0	4.4

[1] First lactation milk yield and initial BW at 21 d before expected calving were covariates for DMI, BW, BCS, and milk yield. First lactation milk fat and CP percentages and initial BW at 21 d prior before calving were covariates for percentages of milk fat and CP, and first lactation milk crude protein percentage and BW at 21 d before expected calving were covariates for milk true protein percentages and milk urea N.

[2] Prepartum dietary RUP.

[3] Prepartum amino acids.
[4] Lactation diet.

TABLE 3. Analysis of variance table for plasma glucose, nonesterified fatty acids (NEFA), growth hormone (GH), insulin and prolactin during the first 70 d of lactation.

		Mean Squares				
Source	DF	Glucose	NEFA	GH	Insulin	Prolactin
Block	8	238.6	12348	6.3	418460	5879
Cov [1]	1	22.4	2476	0.2	75992	11285
BW [1]	1	9.6	175571	15.3	60161	13274
Cov2 [1]	1	273.4	36884	16.7	72251	97
RUP [2]	1	50.0	7432	13.4	93957	19397
AA [3]	1	33.8	15776	0.9	1332	7662
RUP x AA	1	81.6	5253	7.4	8401	18137
LD [4]	1	0.6	18368	0.4	16412	14827
RUP x LD	1	404.6	21557	17.1	13778	7932
AA x LD	1	111.9	904	2.9	2090	15257
RUP x AA x LD	1	239.4	6887	0.7	7867	1725

[1] First lactation milk yield data, initial BW at 21 d before calving, and plasma concentration of glucose, nonesterified fatty acids, growth hormone, insulin, and prolactin in a sample taken 17 d before expected calving were covariates for concentrations of the same metabolites in samples taken thereafter.

[2] Prepartum dietary RUP.

[3] Prepartum amino acids.

[4] Lactation diet.